

# Bioelectricity of non-excitable cells: modeling of instructive multicellular patterns

*Javier Cervera, Michael Levin, Salvador Mafe\**



Universitat de València, Spain, EU

Allen Discovery Center at Tufts University, MA, USA



Universitat de València  
*Established 1499*



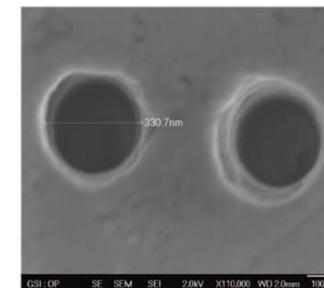
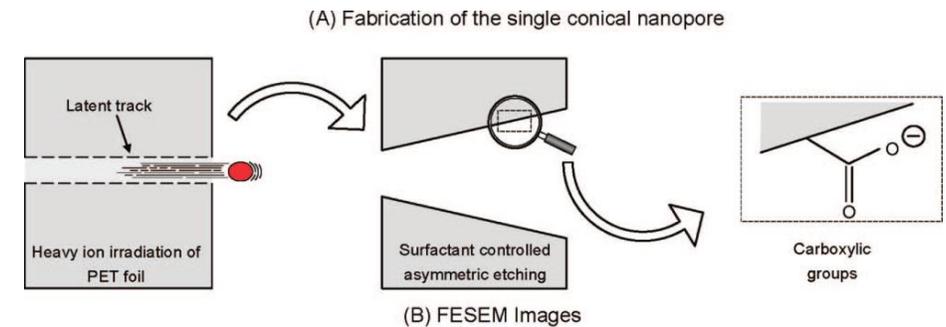
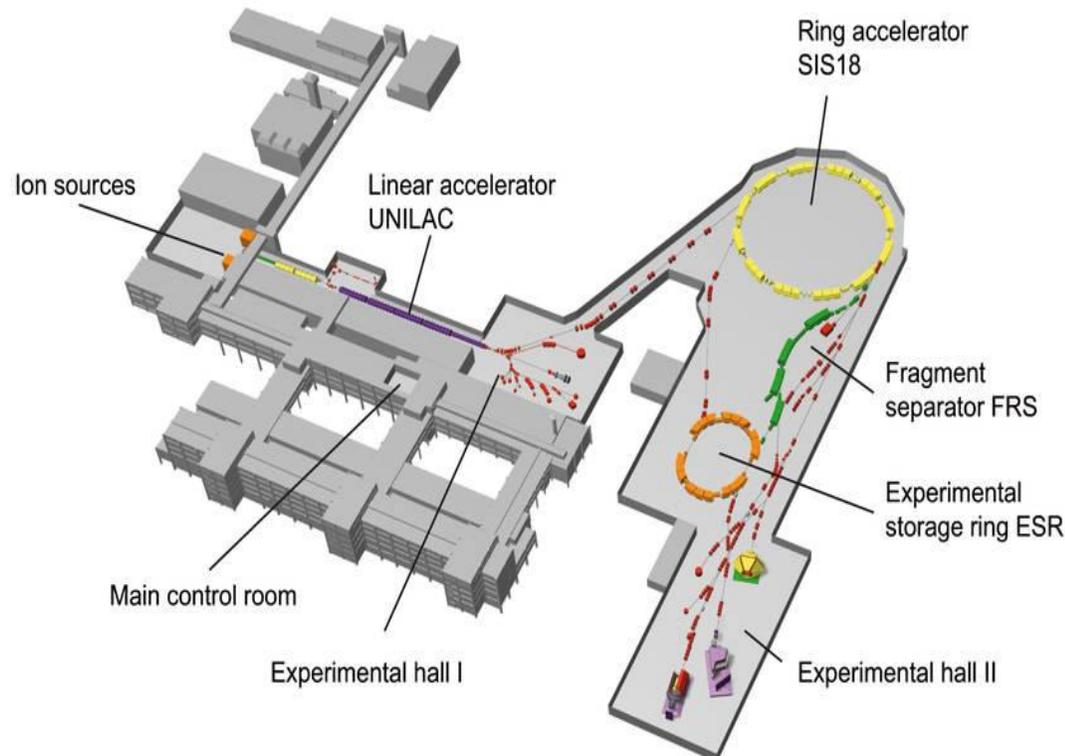
Santiago Ramón y Cajal  
*Catedrático de Anatomía UV, 1883-1887*

1. *Where we (Javier and Salvador) come from*
2. Multicellular organization: bioeng., biochem., and bioelec. views
3. Some experimental facts of bioelectricity + molecular biology
4. Bioelectrical model assumptions and equations
5. Theoretical results of qualitative relevance
6. *Where we go*: identifying key bioelectrical steps in biological complexity

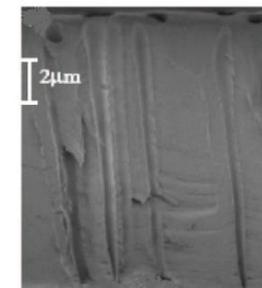
# 1. Where we (Javier and Salvador) come from

## 1a Applied Physics/Nanoscience/Biomimetic nanopores (with Patricio Ramirez)

*Biomimetic nanopores* with voltage-gated conductances and memory effects provide *qualitative insights* on biological channels. A single conical nanopore fabricated by irradiation of a polymer foil (polyethylene terephthalate or polyimide) at the linear accelerator UNILAC with single swift heavy ions (Pb, U, and Au) of energy 11.4 MeV per nucleon and subsequent chemical etching.



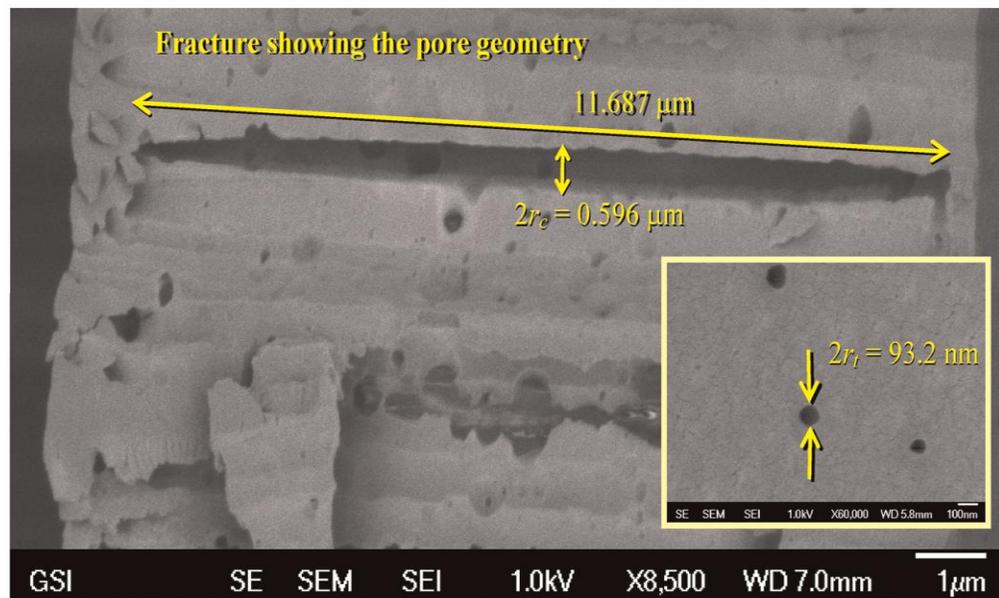
Surface (pore base)



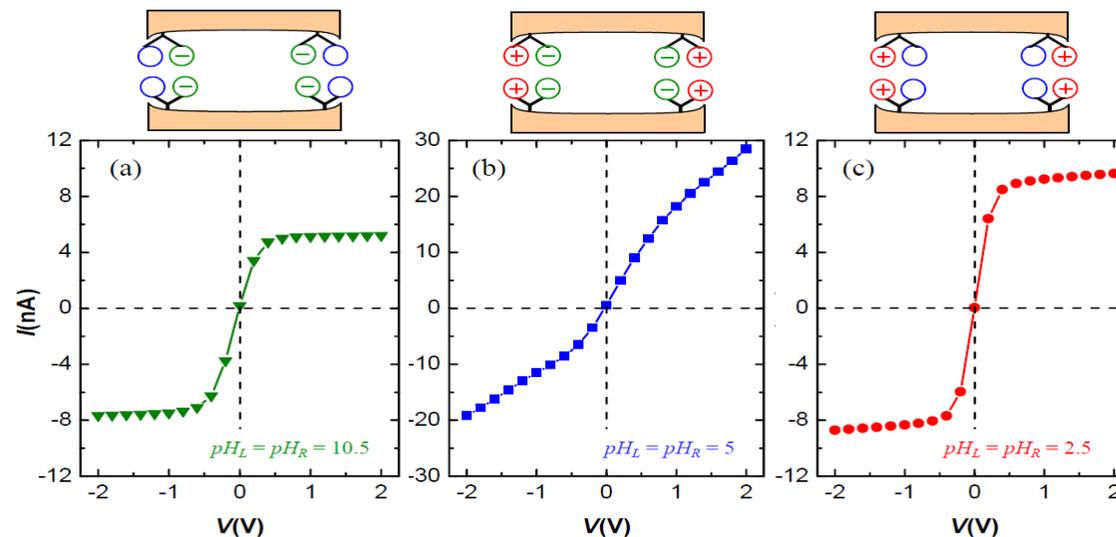
Cross-section

# nanopores as chemically-gated transistors (with M. Ali and W. Ensinger)

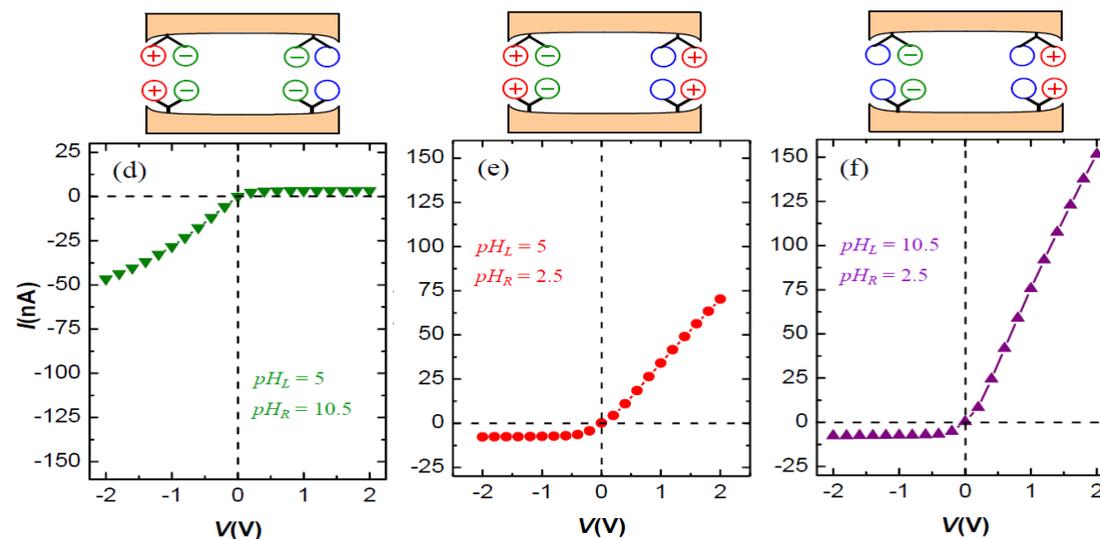
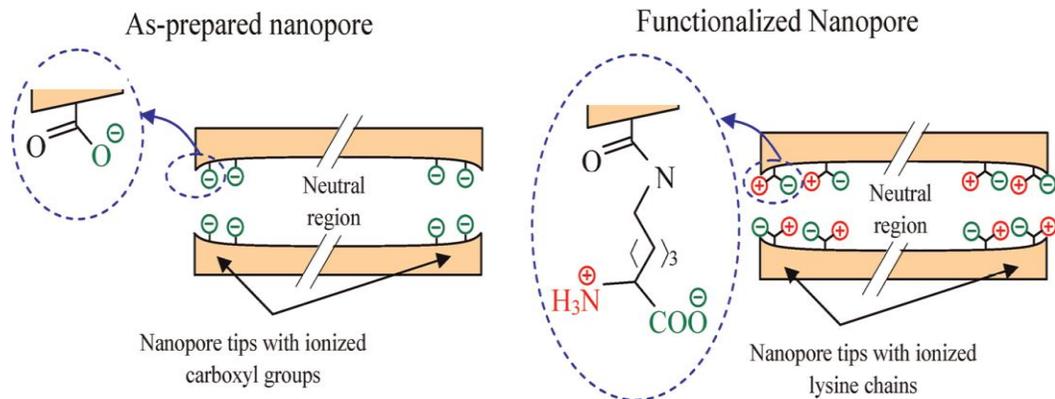
(a) Pore geometry



*I-V* curves of cigar-shaped nanopores functionalized with lysine chains

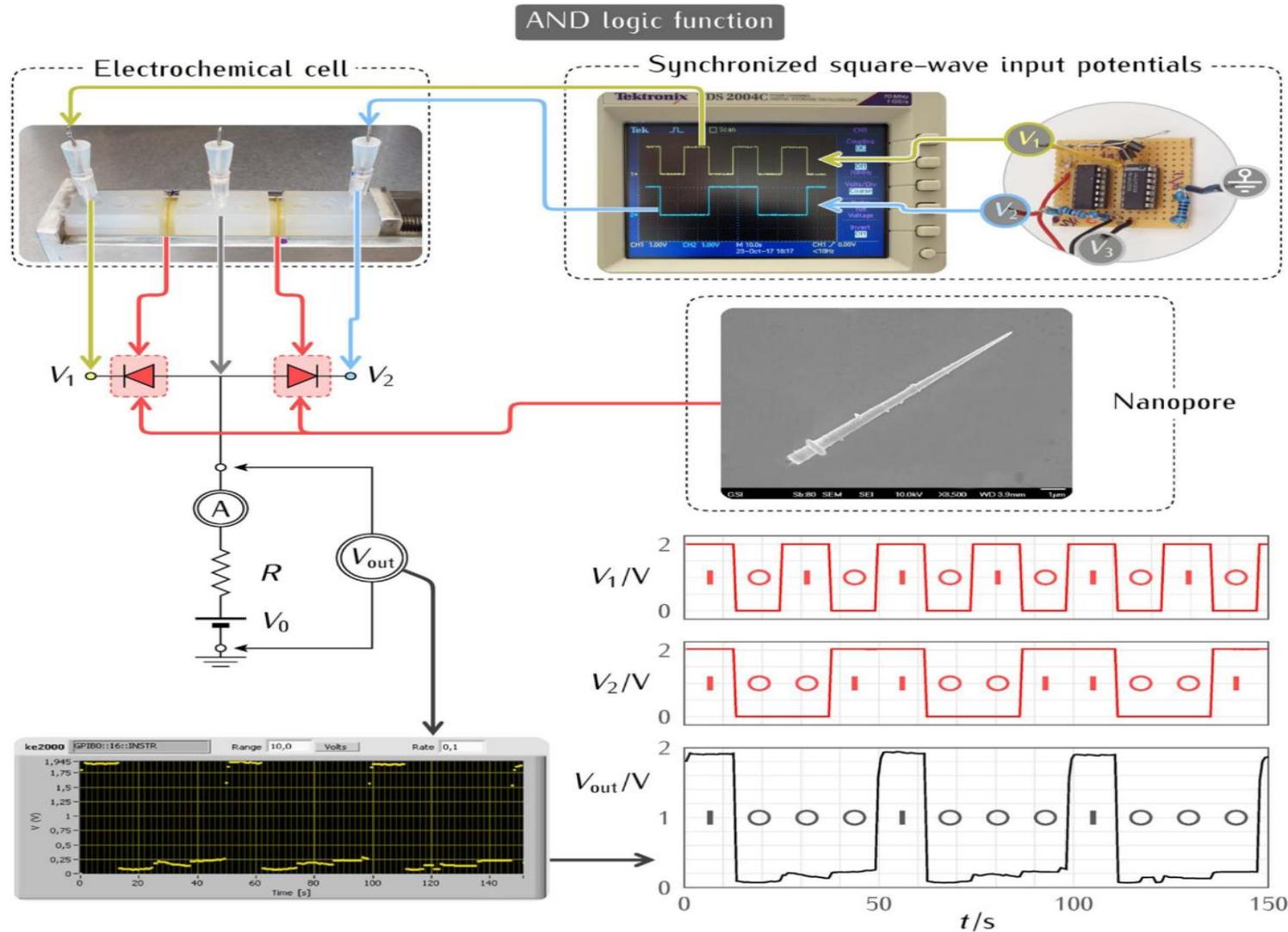


(b) Cigar-shaped pores after functionalization



# nanopore logical functions (with Vicente Gómez)

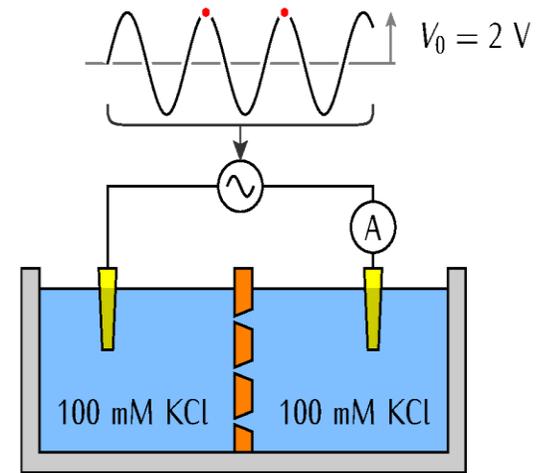
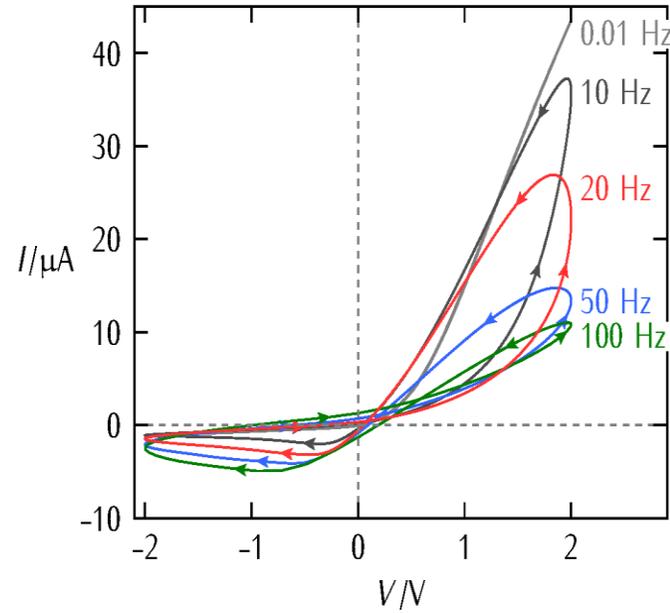
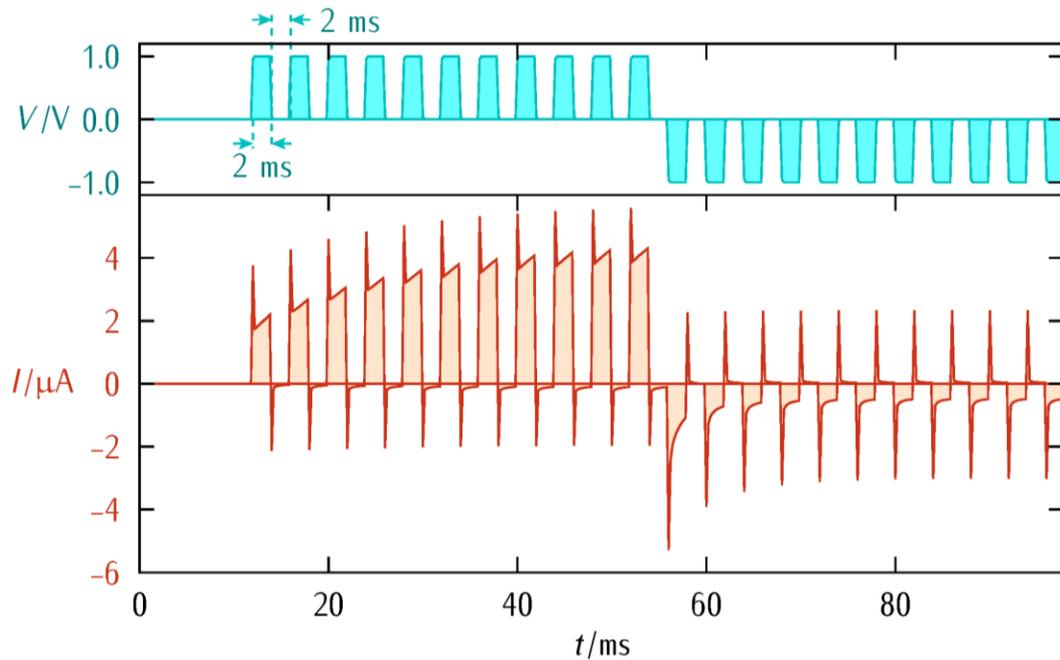
Phys. Rev. Applied 2017  
10.1103/PhysRevApplied.7.064035  
Appl. Phys. Lett. 2012, 2016  
10.1063/1.4754845  
10.1063/1.4954764  
Electrochem. Commun. 2018  
10.1016/j.elecom.2018.01.016



nanopores as rectifying neuromorphic memristors  
(with Juan Bisquert and Sergio Portillo)

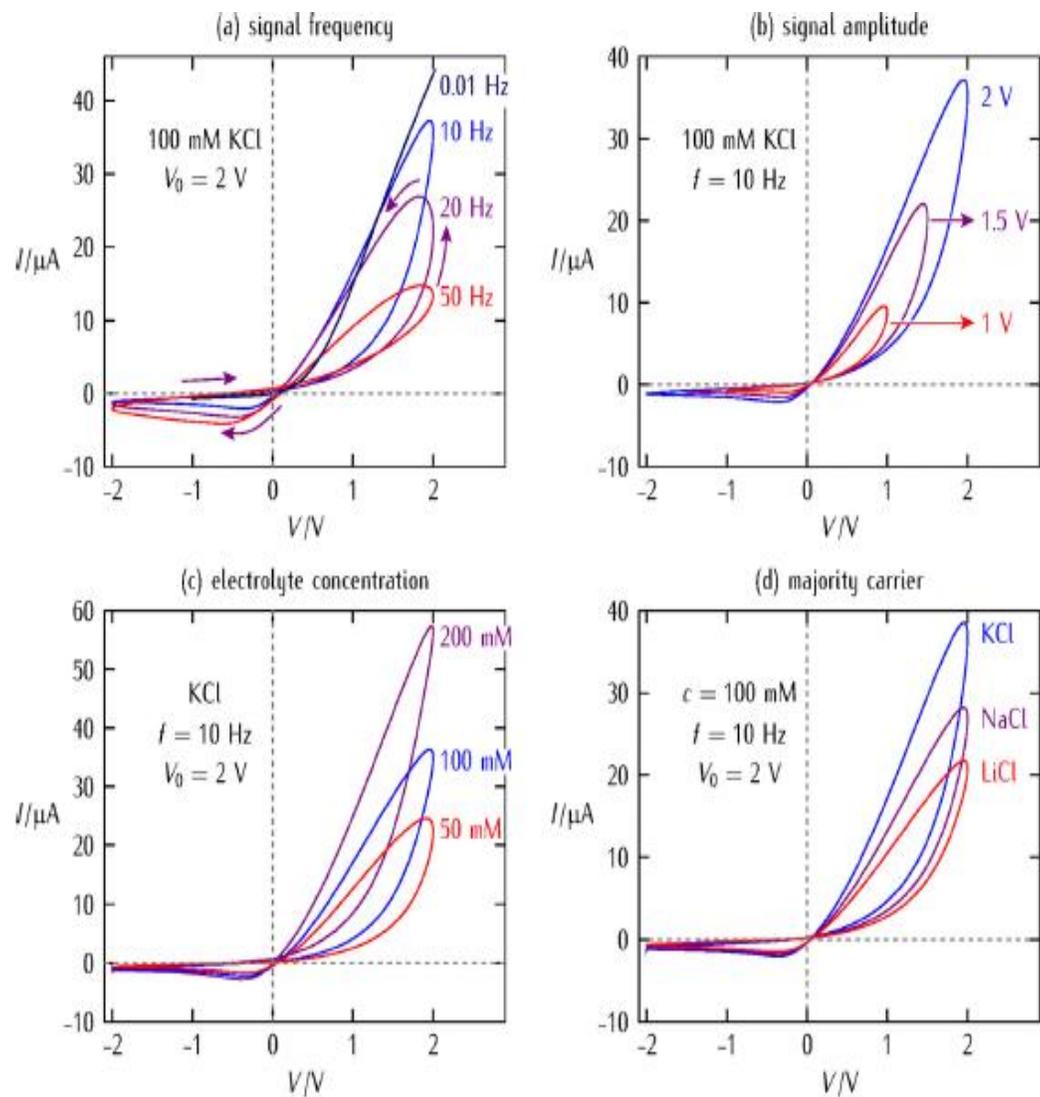
J. Phys. Chem. Lett. 2023  
10.1021/acs.jpcllett.3c02796  
Phys. Fluids 2024  
10.1063/5.0204219

frequency-dependent ionic currents  
and voltage-gated pore conductances

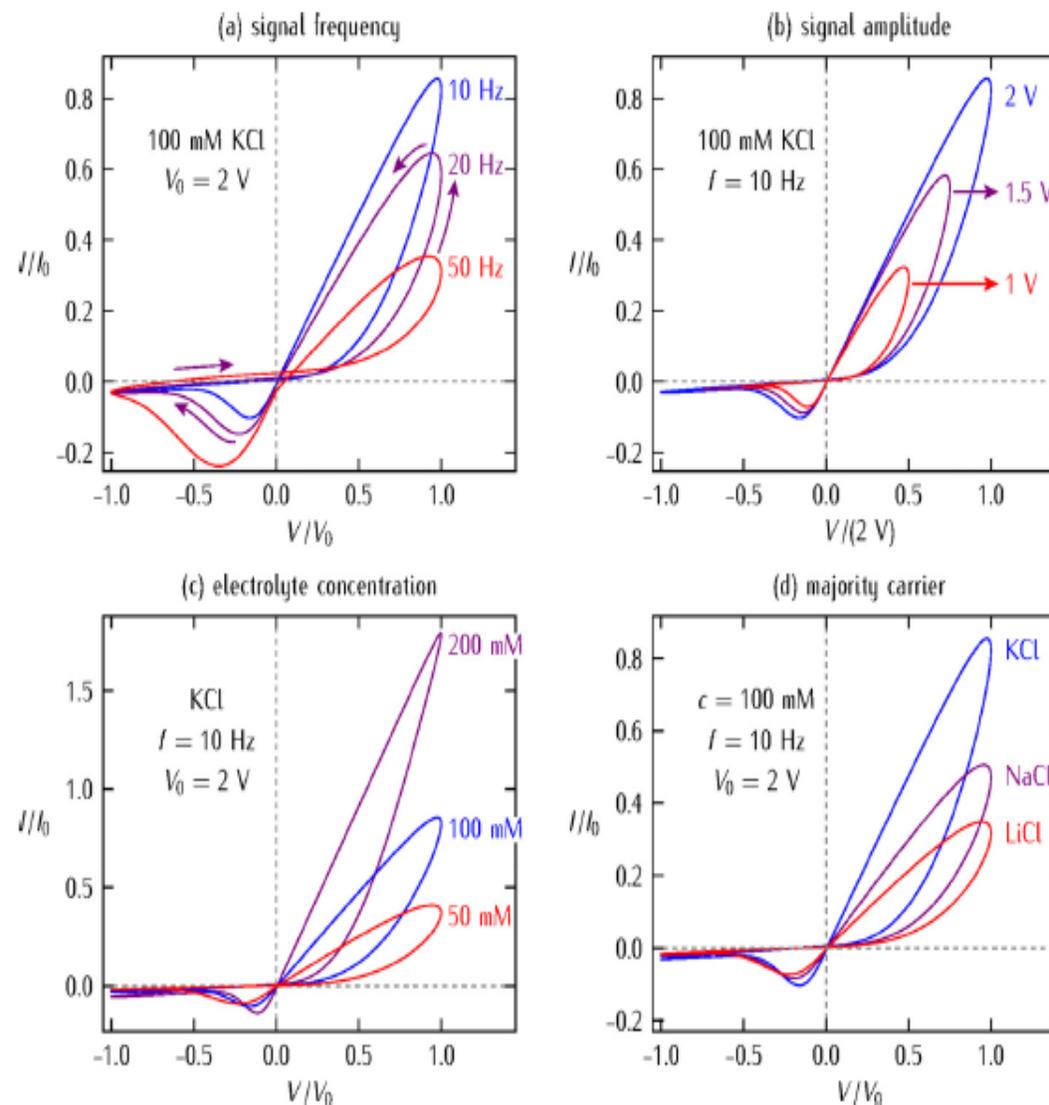


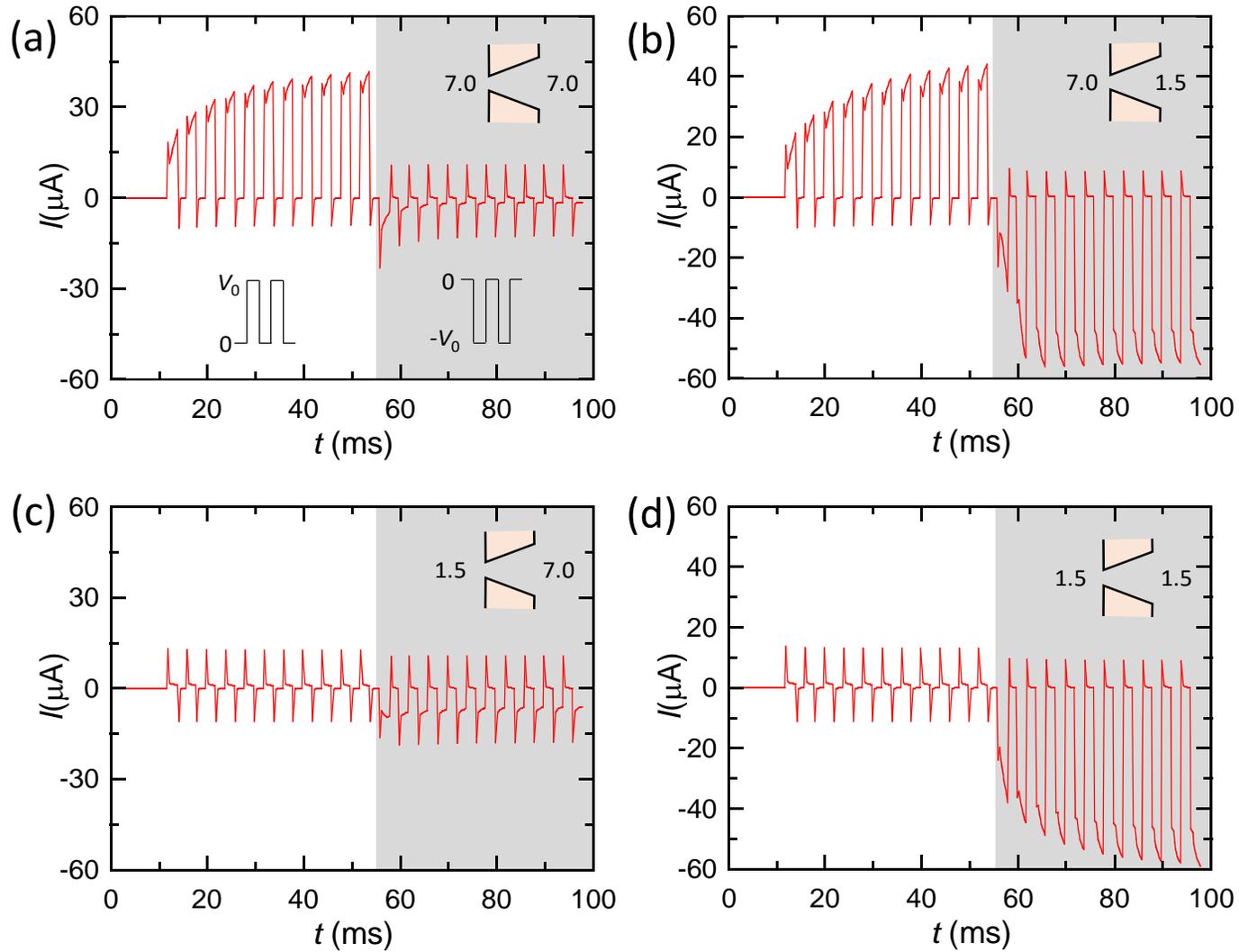
conductance *potentiation/depression*

## Experimental data

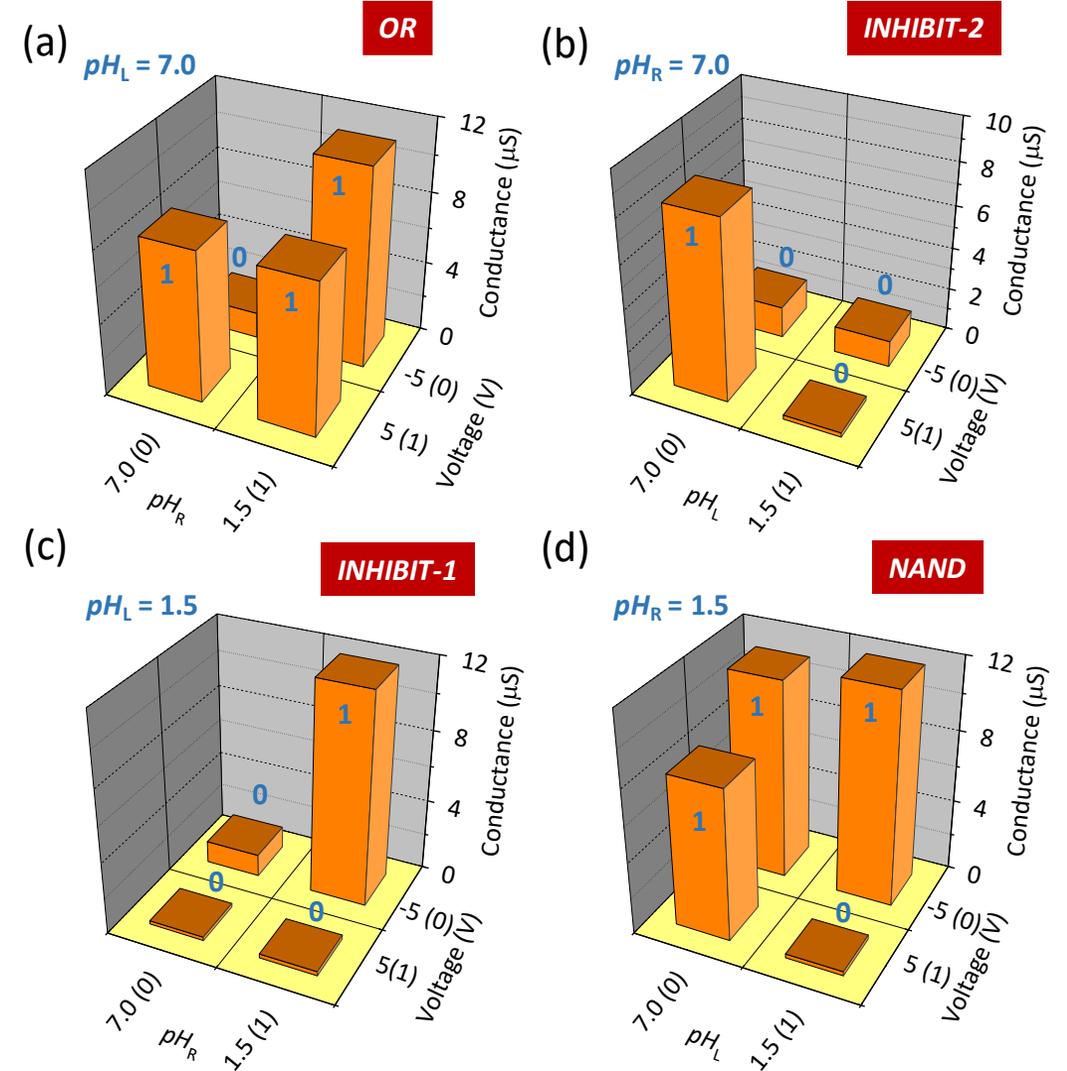


## Model results

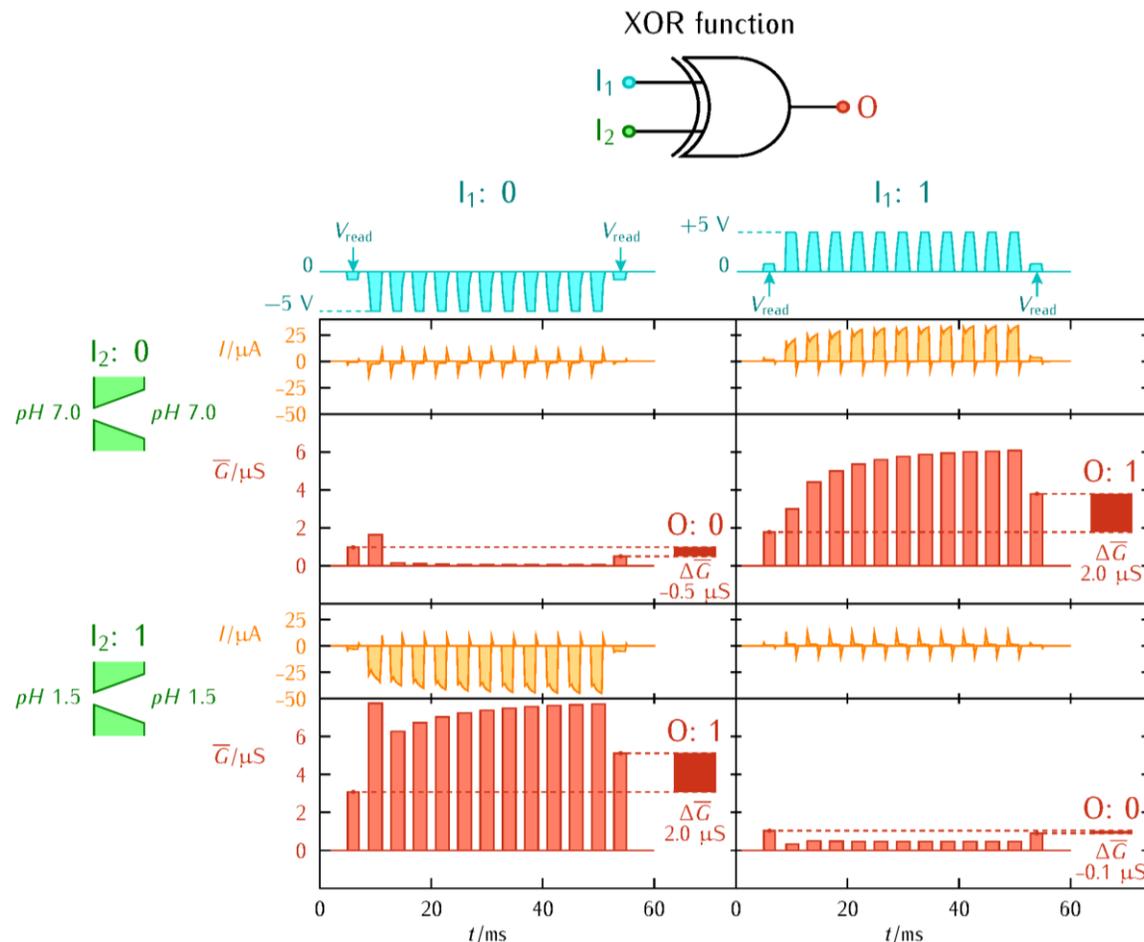




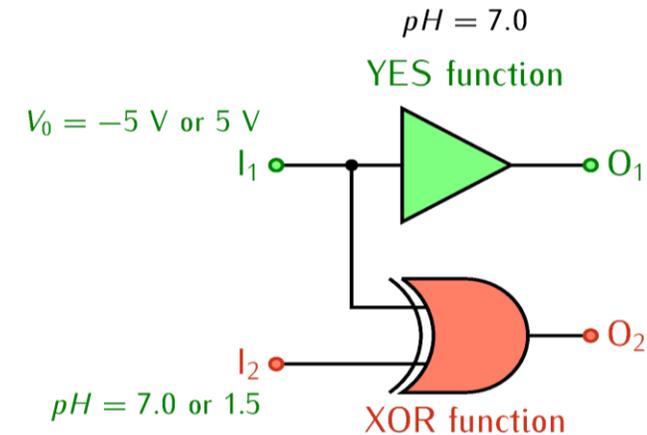
*Current vs. time* traces for different *pH tip/base pore configurations* obtained with sequences of voltage pulses.



*Logic functions*. Inputs *applied voltage V* and *pH*. Output membrane *conductance G = I/V*.

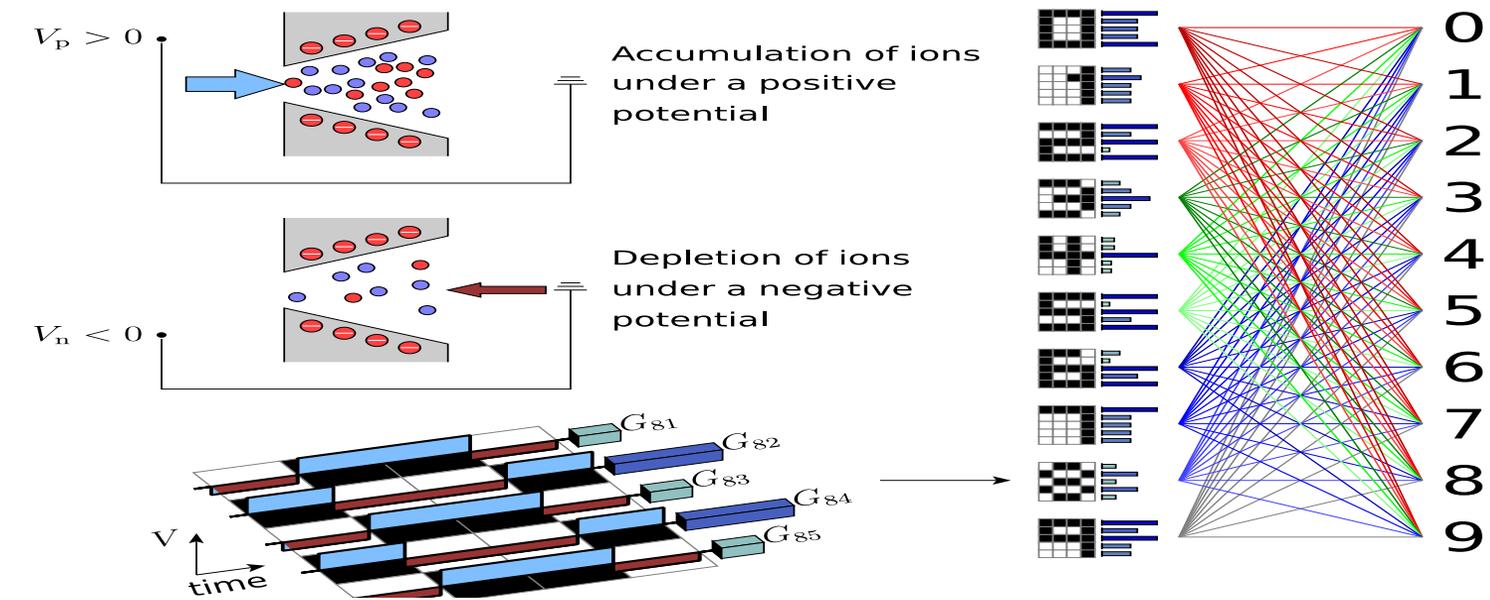


*Voltage sign* and the *pH solution (inputs)* give a *XOR* logics for *membrane conductance (output)* in a 100 mM KCl solution. Sequences of negative and positive voltage pulses give the average conductance difference that characterizes the conductance potentiations.

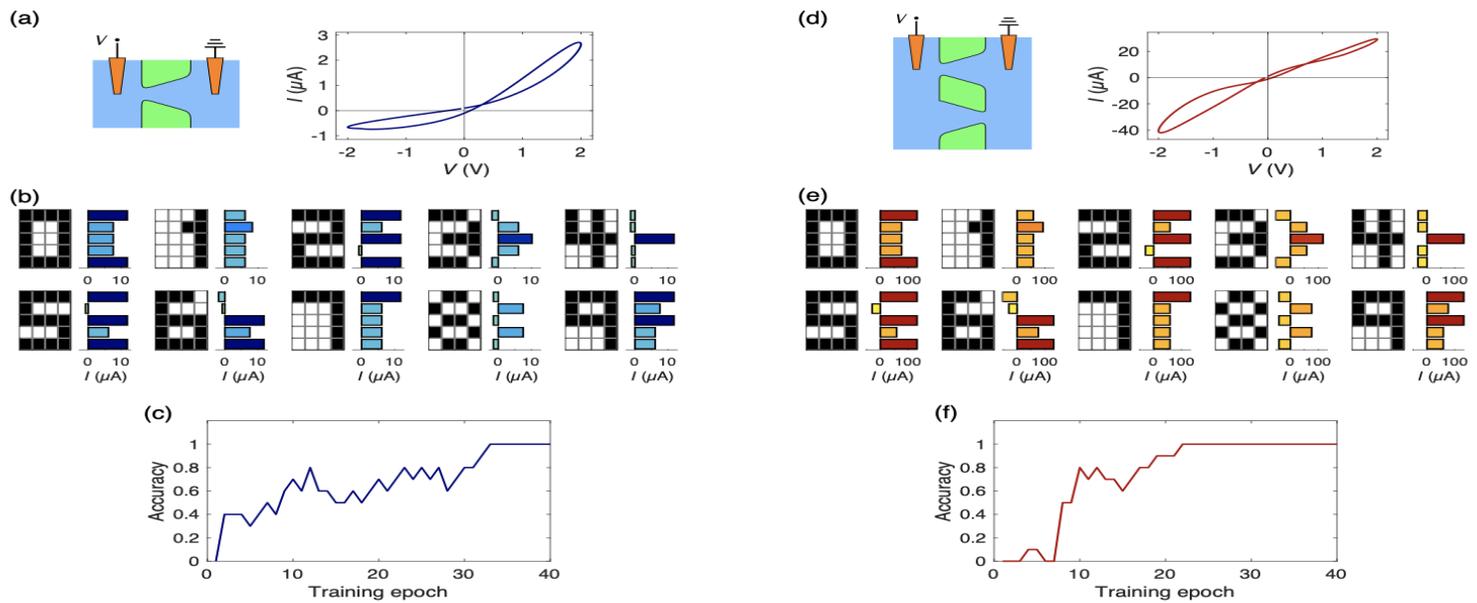


Input		Output: $ \Delta\bar{G} /\mu\text{S}$	
$I_1 \equiv V_0/\text{V}$	$I_2 \equiv \text{pH}$	$O_1$	$O_2$
-5 (0)	7.0 (0)	0.5 (0)	0.5 (0)
+5 (1)	7.0 (0)	2.0 (1)	2.0 (1)
-5 (0)	1.5 (1)	0.5 (0)	2.0 (1)
+5 (1)	1.5 (1)	2.0 (1)	0.1 (0)

*Feynman reversible logical function* implemented by the combination of the *YES* and the *XOR* functions.



Mapping of *positive and negative voltage pulses* (input) into the distinct reservoir states (read-out) identifies *patterns of ten digits* (output). Ionic accumulation (voltage  $V_p > 0$ , high membrane conductance) and depletion (voltage  $V_n < 0$ , low membrane conductance) in the *nanofluidic conical pores* provide the *short-term plasticity* of the membrane conductance.

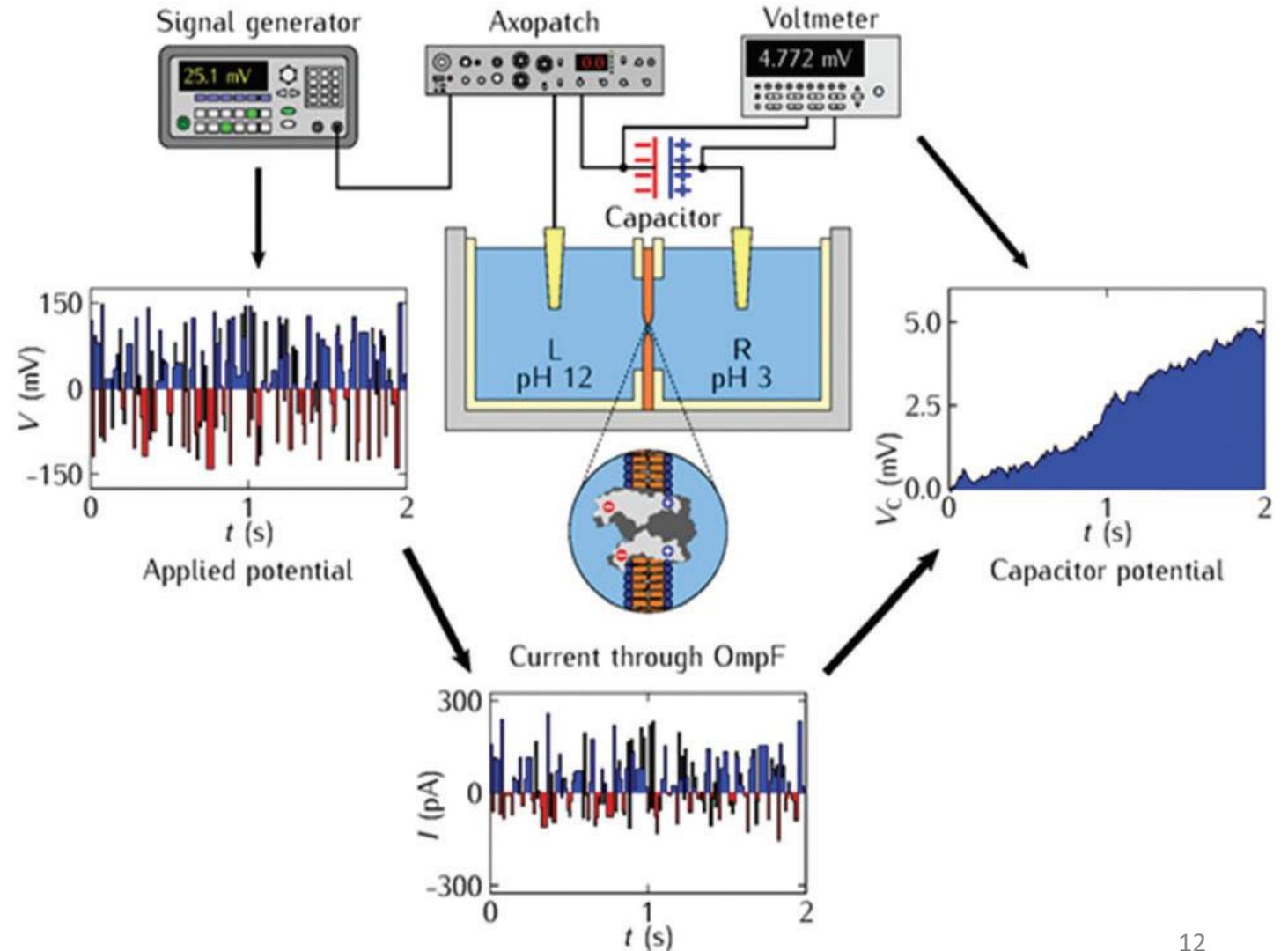
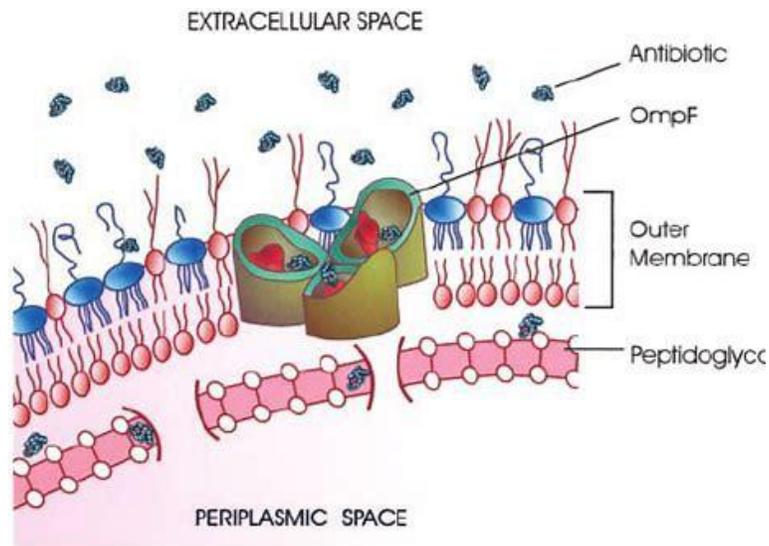


Final conductance states for the digits  $i = 0, 1, \dots, 9$  and the membranes  $j = 1, 2, \dots, 5$  are grouped in *conductance matrix*  $G_{ij}$ . Reservoir states are then processed by the read-out layer, which associate the different pulse inputs patterns to the distinct digit outputs. The *accuracy vs. training epoch* curves.

# 1b Molecular biophysics: protein channels as nanofluidic diodes (with Patricio Ramirez)

Small 2018  
10.1002/sml.201702252  
Phys. Chem. Chem. Phys. 2017  
10.1039/c6cp06035h

Electrical rectification produces cumulative effects in the Outer Membrane Porin F of *E. Coli* inserted in an artificial lipid bilayer



## 2. Multicellular organization: bioeng., biochem., and bioelec. views

In *biological patterns*:

- individual cells can make decisions with *local info*, usually without having a highly precise knowledge of its particular position in a multicellular ensemble;
- *environmental changes* and *individual variability* may help *system exploration*. The result is a continuous interplay between *short-term* individual changes and *long-term* collective persistence; and
- traditional attempts to understand *instructive patterns* have favored spatio-temporal distributions of *biochemical* signals.

## 2.a Distributed epigenetic shape formation and regeneration algorithm for a swarm of robots

**Algorithm 2** Algorithm for the shape regeneration by the swarm of robots

```
1: while TRUE do
2:   if State == Danger then
3:     LId == LeaderElection(); {the robot has the lowest Id
      among all the Queued robots}
4:     if Id == LId then
5:       State ← Leader;
6:     else
7:       State ← Queued;
8:       Algorithm-1();
9:     end if
10:  end if
11:  if State == Leader then
12:    CountPopulation(); {Count the remaining population}
13:    GenerateScaledShape(); {Scale down the input target
      shape}
14:    Share the new shape with the remaining robot;
15:    State ← Active;
16:    Form new seed robots;
17:    Algorithm-1();
18:  end if
19: end while
```

GECCO '18 Companion 2018 (ACM)  
10.1145/3205651.3208300

Biologically inspired algorithm for shape formation and regeneration exhibited by a swarm of robots.. Here, a *gene* is similar to an *instruction manual* which aids cells to *reproduce and form* arbitrary shapes. Given an input binary image, the gene required for the shape formation is computed and transferred to *all the robots* constituting the swarm. The robots (“cells”) then use this gene to form a shape in a distributed and decentralized manner.

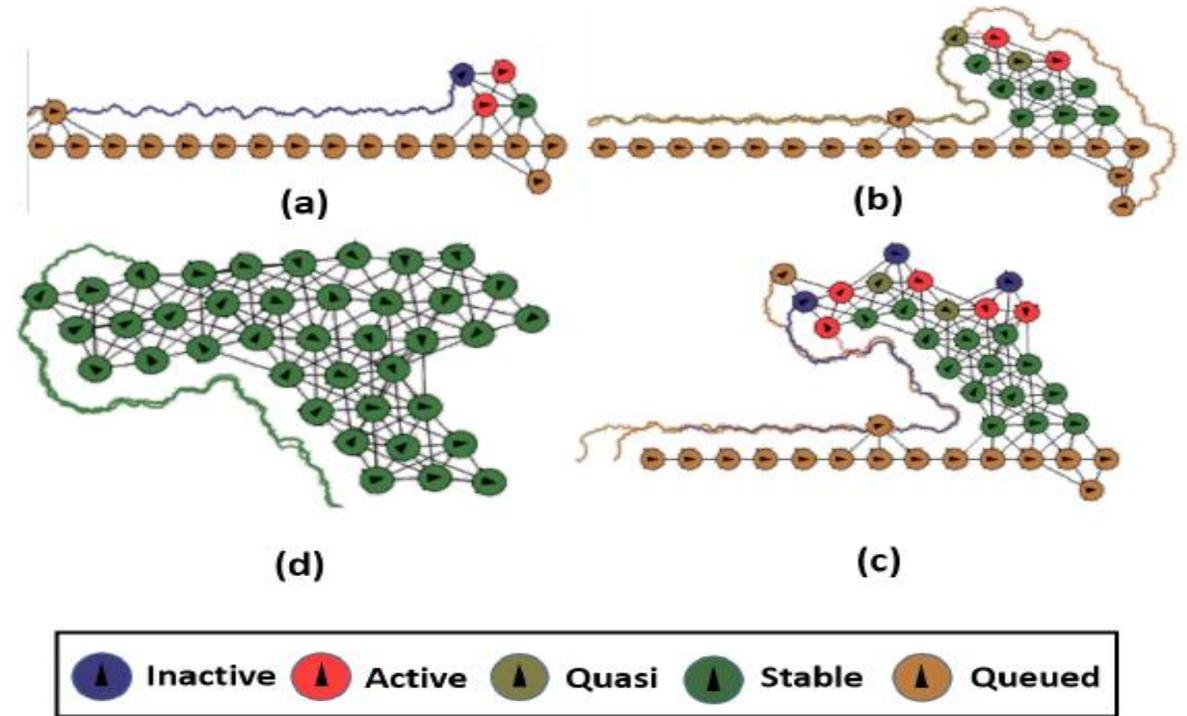
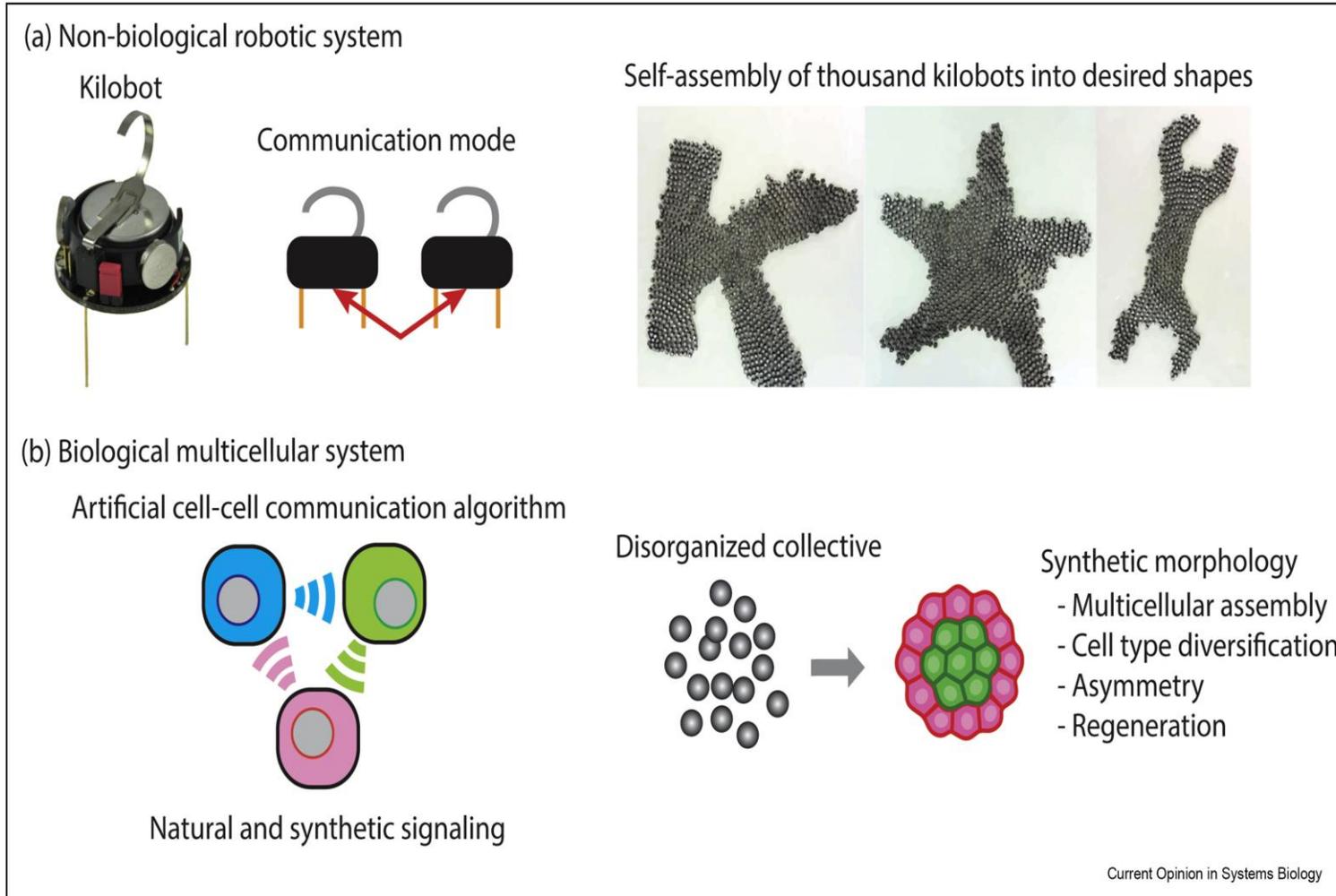


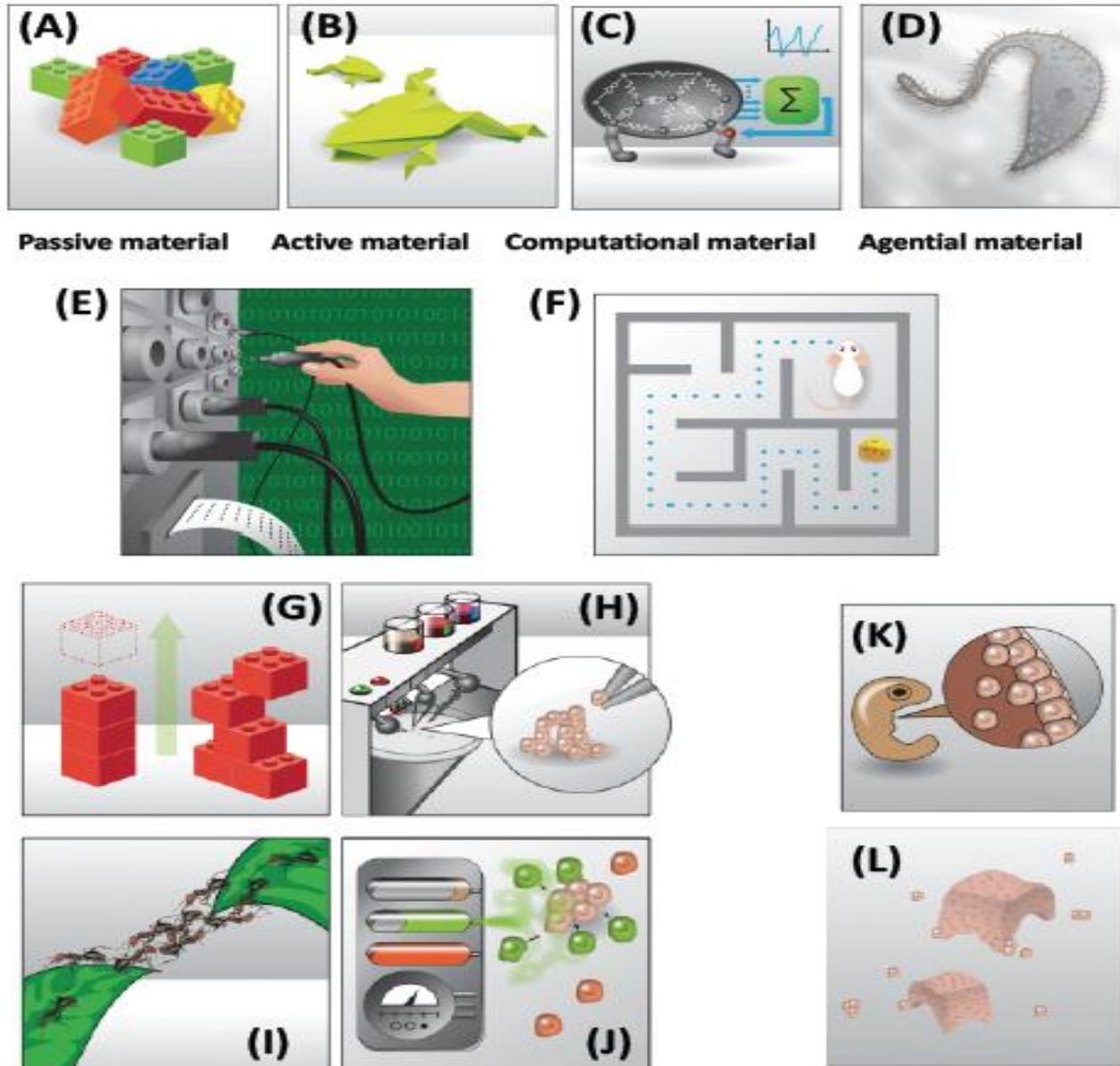
Figure 5: Generation of alphabet ‘T’ in 2 hours 50 minutes and 8 seconds

## 2.b Forward engineering of collective systems



(a) *Robotic swarms of Kilobots* can communicate with neighbors by reflecting infrared light off the table below to decide how they move according to a *user-designed communication algorithm*. Examples of collective patterns.

(b) *Engineering of cell–cell interactions to drive multicellular self-organization*. Artificial algorithms can program disorganized cells to behave collectively and self-organize into particular structures. Features can mimic processes in natural developmental systems (self-assembly, cell type diversification, symmetry breaking, and regeneration).



Engineering with passive materials (A) managed for desired functionality has moved toward active matter (B) and computational media (C). Biorobotics considers material as *agential*, composed of subunits (D, living cells) that include *problem-solving* in *problem spaces*. They are not only subjected to rewiring (E) but also to behavior-shaping (F). In complement to 3D-printing designed for building with passive matter (G), which also works with cells (H), collective behavior of living systems, e.g. ant swarm (I), allows manipulating the collective behavior of cells (J) in *anatomical morphospace*, as instructive signals from other cells cause frog ectodermal cells to be a 2-dimensional barrier in embryos (K) and stimuli can achieve guided self-assembly toward novel form and function (L).

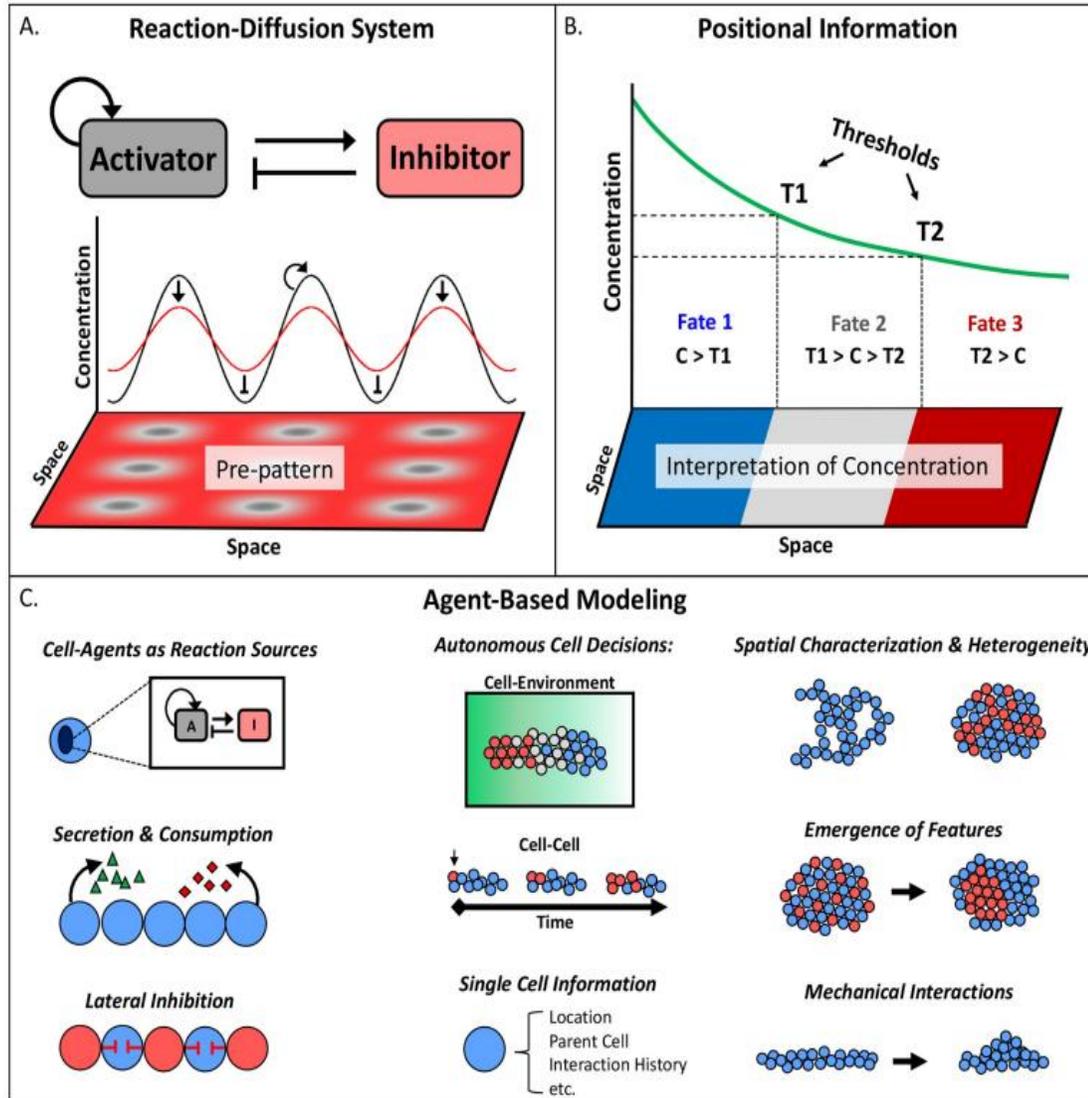
## 2.d Biochemical multicellular patterns

### pattern formation during morphogenesis

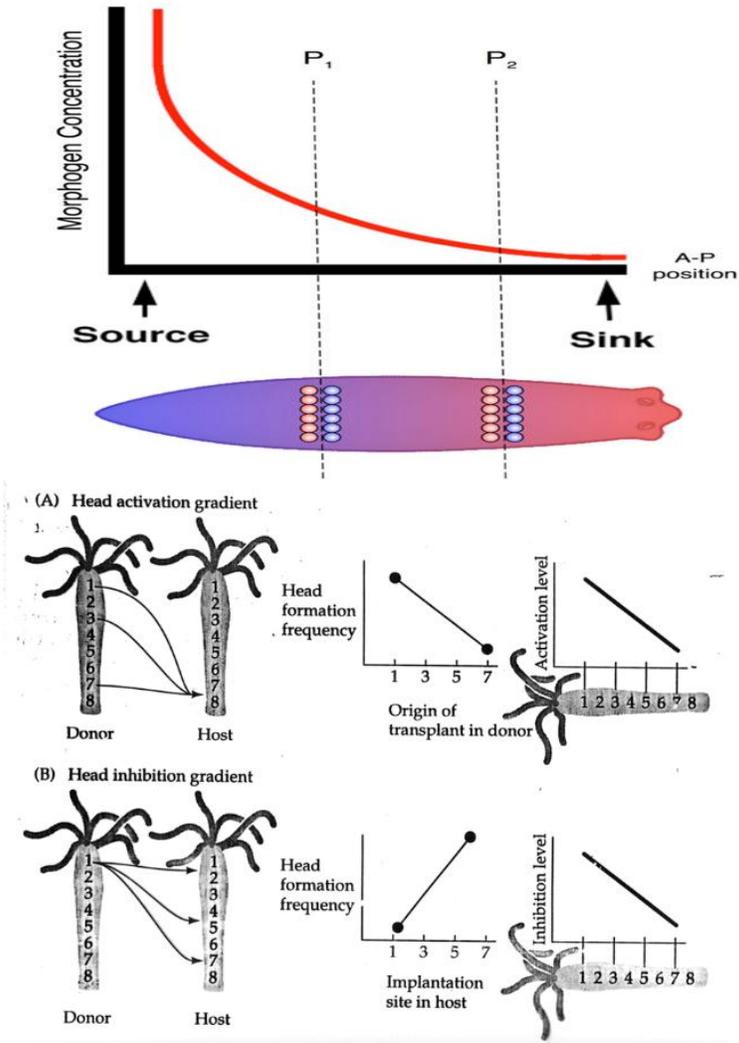
(A) *Turing reaction–diffusion system* with an activator and an inhibitor: a chemical prepattern is established in advance of cell fate decision.

(B) *Wolpert’s positional information*: concentration thresholds allow cell multiple fate decisions from a single molecular gradient.

(C) *Agent-based models* incorporate features from both theories. Cell agents are sources of activators and inhibitors, permit localized reactions, and make autonomous decisions in response to local environment. Static and dynamic spatio-temporal patterns can reproduce complex behaviors.



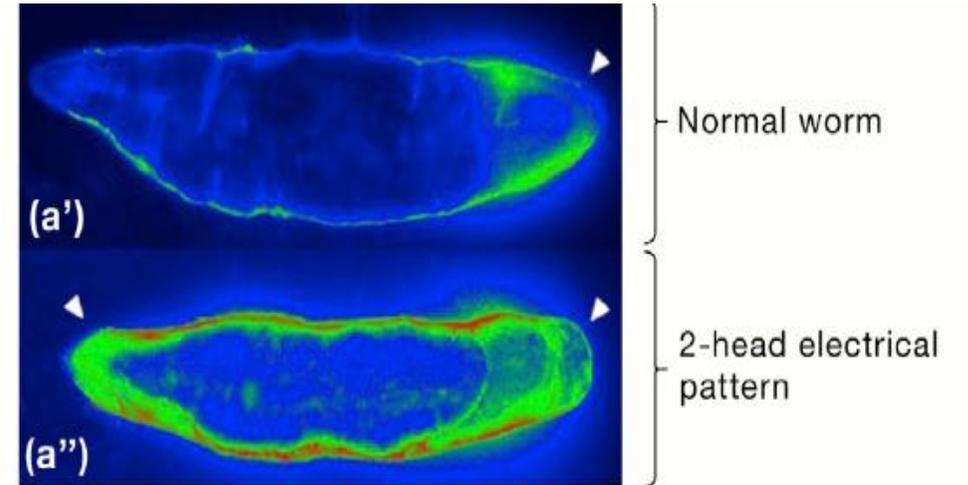
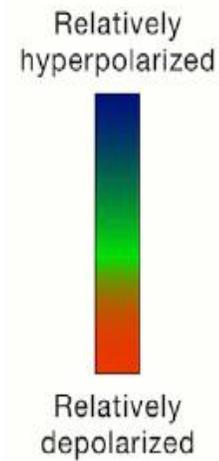
## Biochemical gradient



one (two?) *morphogen gradients* needed  
(a head activator and a head inhibitor?)

vs.

## Bioelectrical pattern

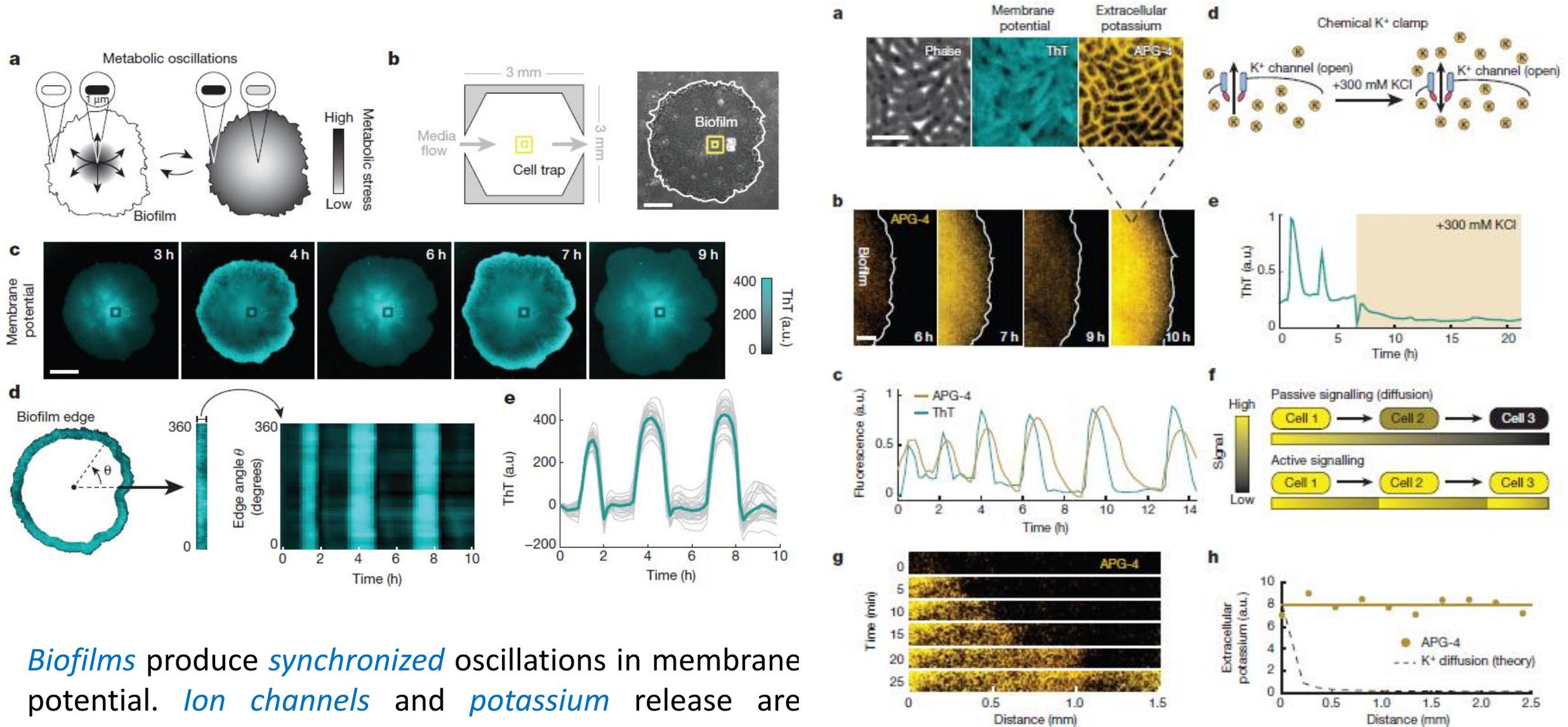


*biofield level -*  
(no head)

*biofield level +*  
(head)

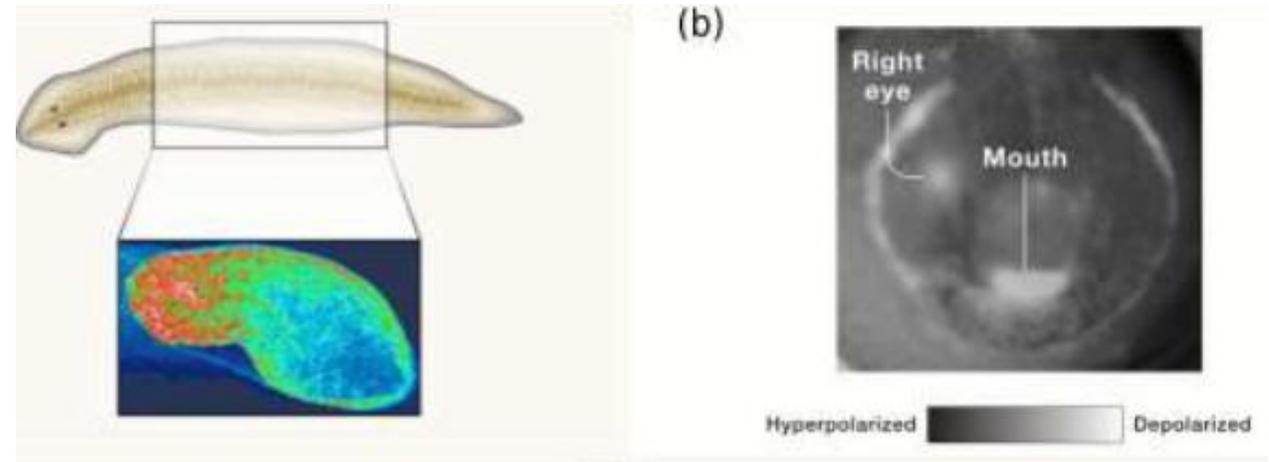
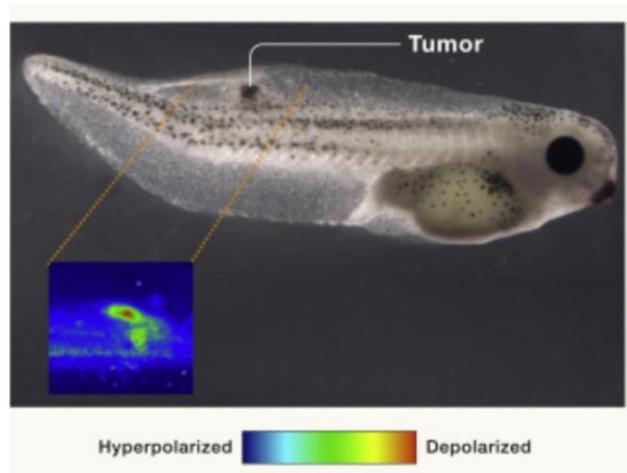
one *bioelectrical field*  
influences the animal polarity

# bioelectrical and biochemical coupling in bacterial communities



*Biofilms* produce *synchronized* oscillations in membrane potential. *Ion channels* and *potassium* release are involved in active signal propagation within the biofilm.

- 1) Cells are sensitive to *spatio-temporal bioelectrical patterns* in *developmental phases* and *regeneration stages*. Cell potential maps can be correlated with distributions of potassium and calcium ions, neurotransmitters, and other transcription activators, acting as *pre-patterns* for slow *biochemical signals*.



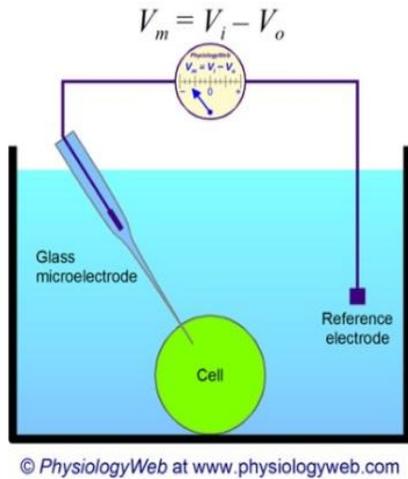
- 2) A *cell* needs both individual *independence* to respond to external stimuli and *coordination* with the multicellular aggregate for morphological outcomes. Information concerning the body plan must reside within the *DNA*, a *single-cell language* for making proteins. However, while morphology is data-driven, it is *not evident* only from the data. Here, we consider the *ion channels* and the *intercellular junctions* as the *computational* and *cognitive* layer needed to specify multicellular coordination.

- 3) We explore a *bioelectrical multicellular language* based on two assumptions:
- 3.1) “*hardware*”: *transcription* provides voltage-sensitive *ion channel* and intercellular *gap junction* proteins that regulate the *cell membrane potential*. In turn, the cell potential allows *feedback* mechanisms that modulate the *transcription* of these proteins;
- 3.2) “*software*”: the spatio-temporal *pattern* of *membrane potentials* constitutes a *bioelectrical network* that contributes to the required *multicellular coordination*; and
- 4) we attempt to reduce biological complexity by identifying a small number of observable magnitudes that may control *crucial steps*, making emphasis on *bioelectrical mechanisms*. Then, we suggest *operational actions* based on *average multicellular potentials*. These *actions* are not only *system-* but also *context-dependent* because of multiple *single-cell* and *multicellular* bioelectrical feedbacks.



### 3.a Single-cell potentials: the membrane as a bioelectrical control surface

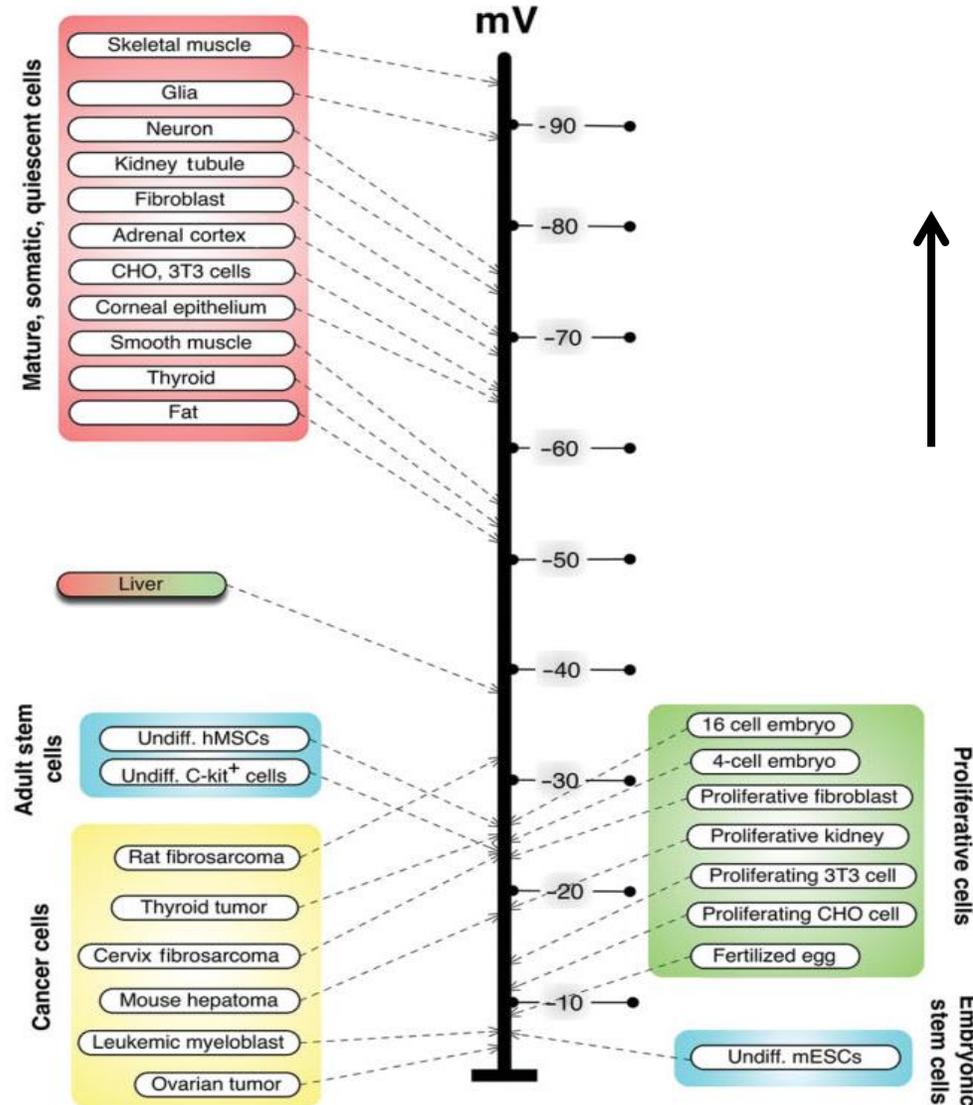
Wires. Sys. Biol. Med. 2013  
 10.1002/wsbm.1236  
 Prog Biophys Mol Biol. 2016  
 10.1016/j.pbiomolbio.2016.06.006  
 PLoS. Biol. 2025  
 10.1371/journal.pbio.3003052



*Polarized* and *depolarized* potential *windows* exist at the individual *cell scale*.

However, studies addressing the *molecular mechanisms* between cell potential and proliferation are lacking.

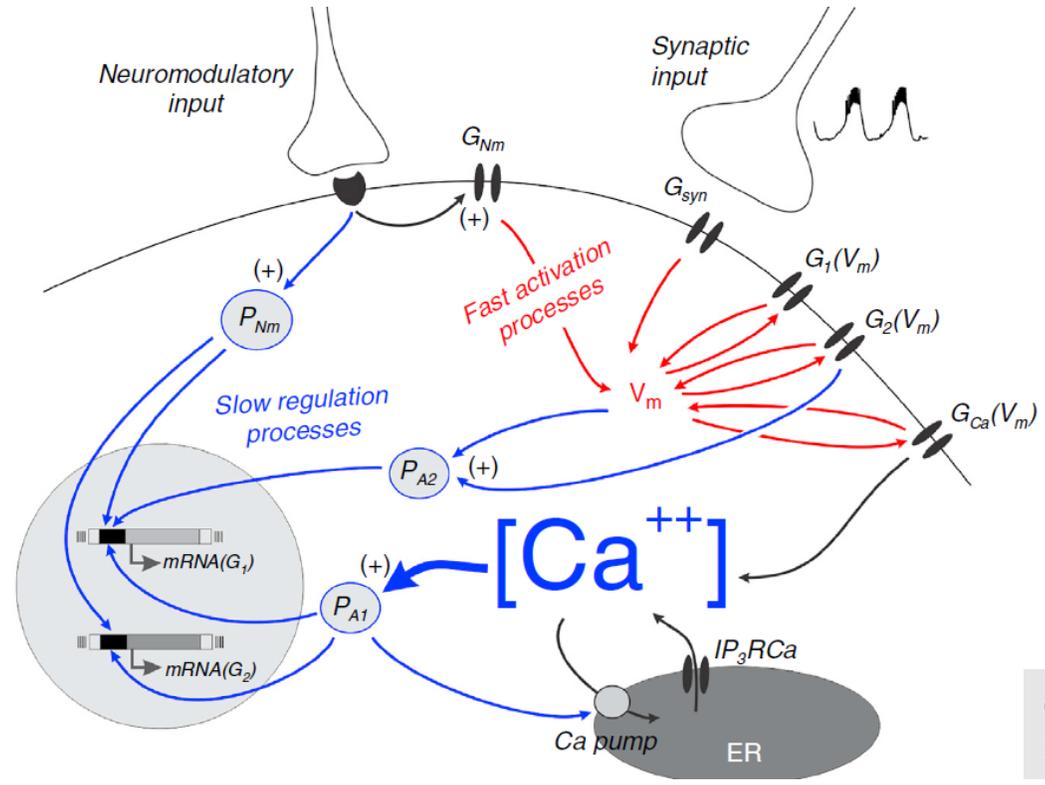
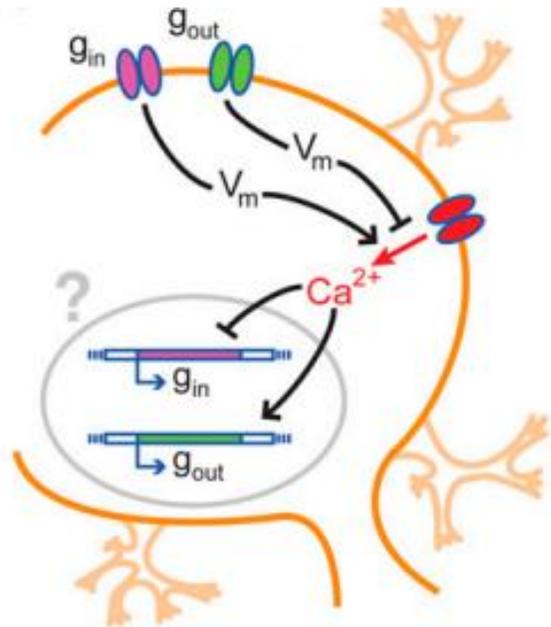
Here, we are interested in *multicellular patterns* and *collective mechanisms*.



Mature cells are *polarized* (high  $|V_m|$ ).  
 The *default* mode?

Proliferative cells are *depolarized* (low  $|V_m|$ ).

### 3.b A reduced number of counteracting voltage-gated channels influence protein transcription, regulating single-cell and intercellular potentials



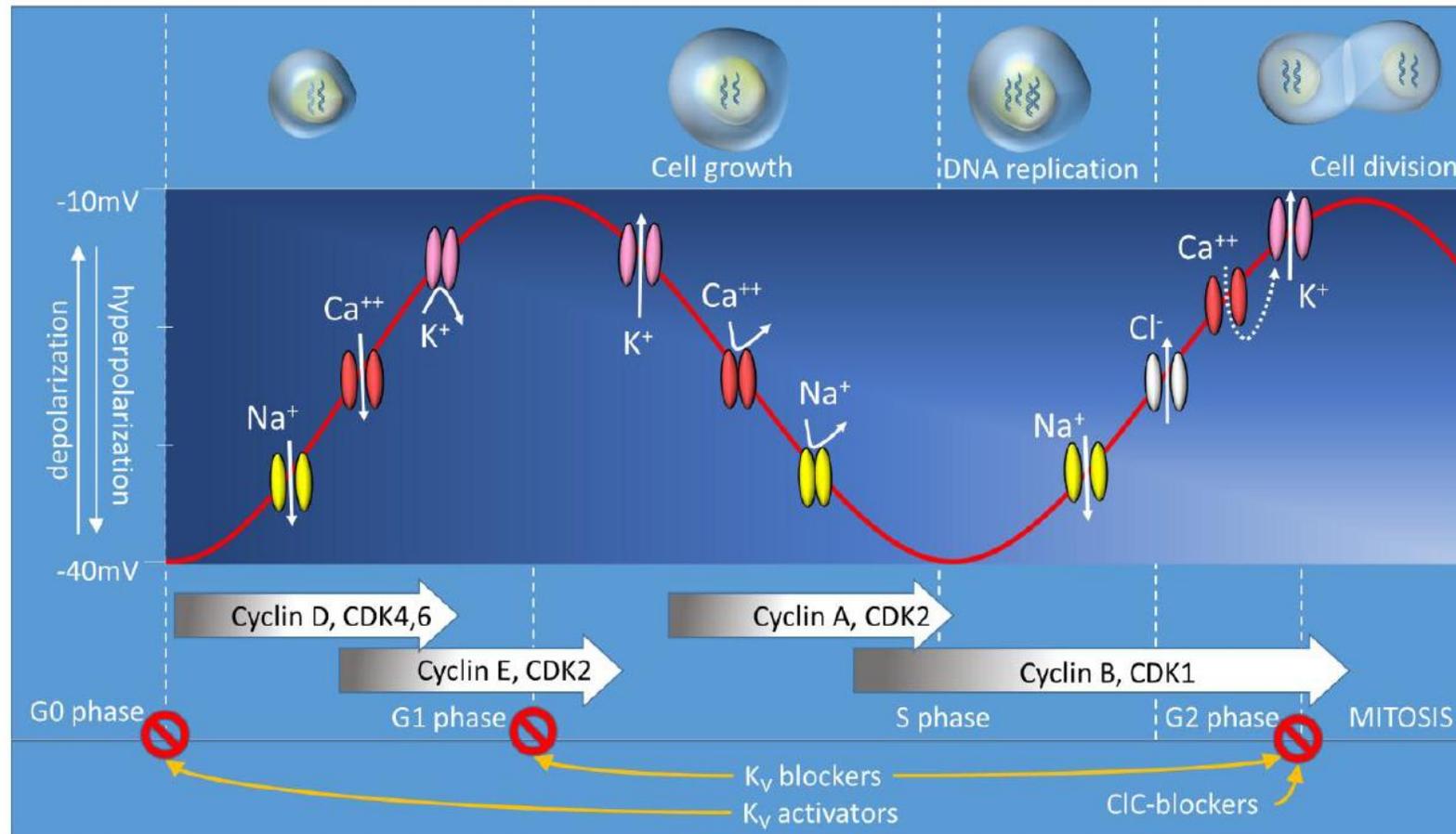
homeostatic channel regulation in neurons

PNAS 2013  
10.1073/pnas.1309966110

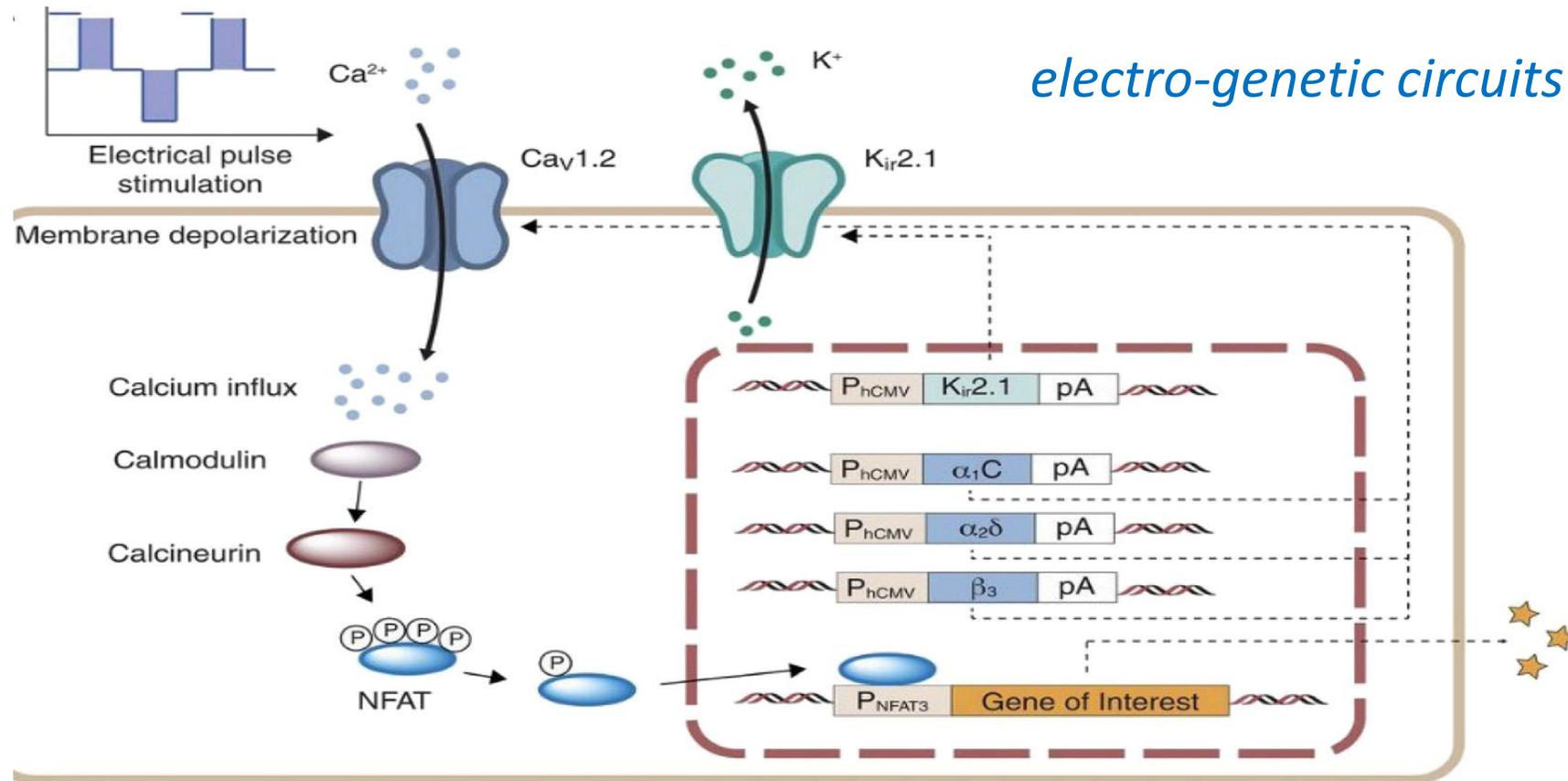
Current Biol. 2019  
10.1016/j.cub.2019.05.029

*Interplay* between *bioelectricity* and *transcription*: voltage-gated channel proteins have mRNA levels modulated by a *calcium-dependent transcription*. A neuron regulated by inward ( $g_{in}$ ) and outward ( $g_{out}$ ) conductances where the  $Ca^{2+}$  influx through voltage-gated calcium channels depends on the membrane potential, which is regulated in turn by the channel conductances.

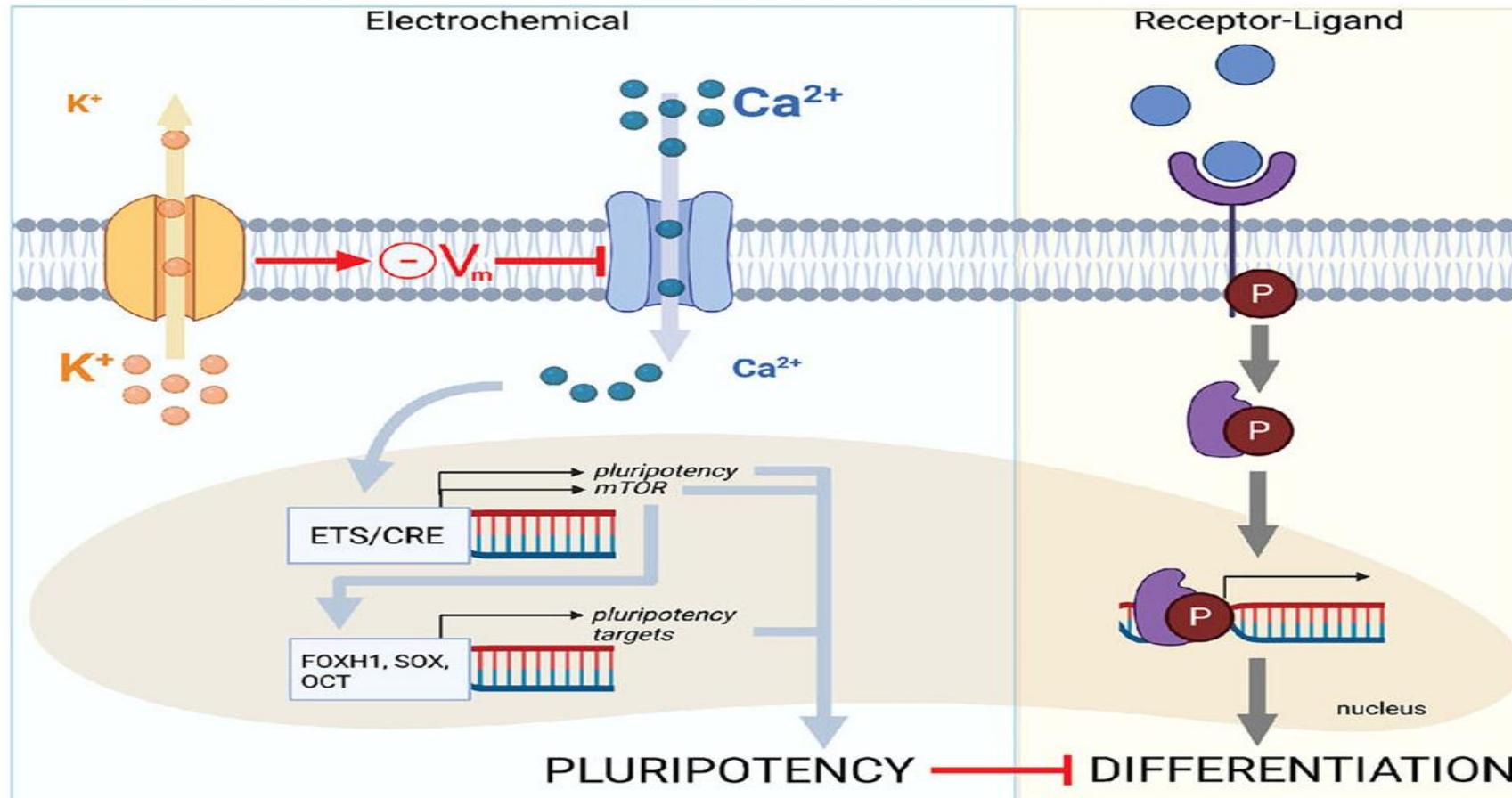
# cell cycle



Proposed model of voltage-gated ion channel activity during the *cell cycle*. Note the *counteracting channel actions* based on their different expression levels. The oscillation of the membrane potential follows the progression of the cell cycle. Could this *bioelectrical process* be arrested at particular *polarized* or *depolarized* cell states by acting on specific channels?



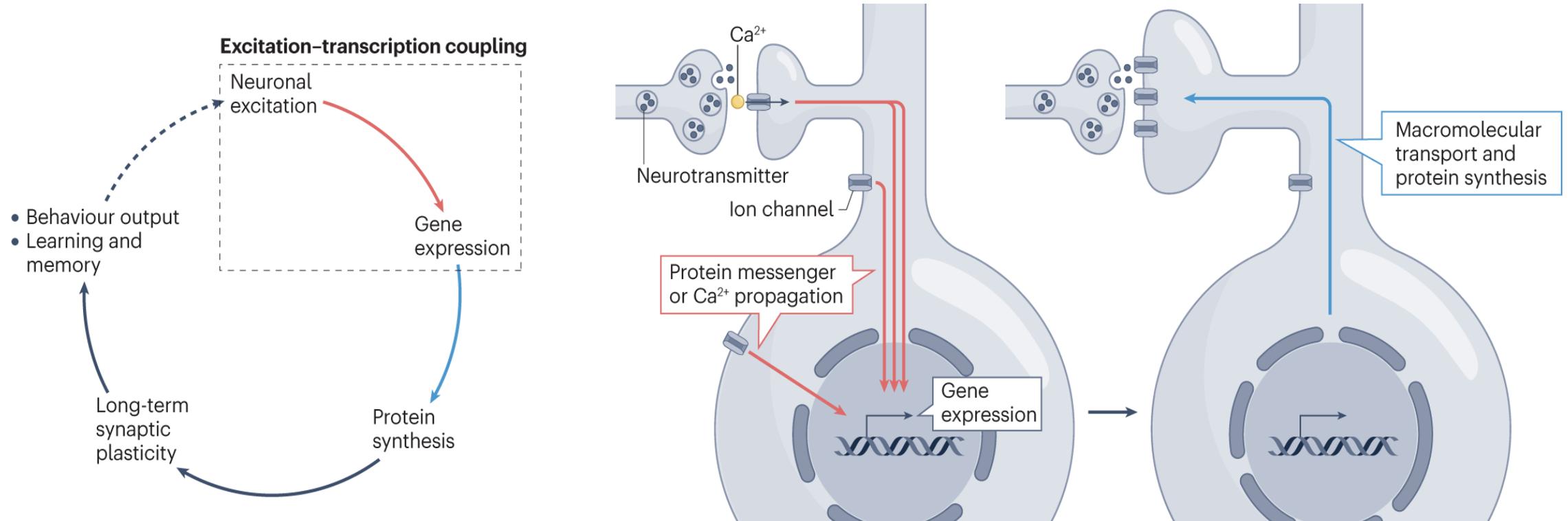
*Transcriptional control of mammalian cells* by membrane depolarization shows the *coupling* between *bioelectrical* and *biochemical signals*. In the electrogenetic circuit, an inward-rectifying potassium (*Kir*) channel sets initially the potential of human embryonic kidney (*HEK-293T*) cells. External pulses provoke the depolarization that opens the voltage-gated calcium channel (*Cav*). The increase in the calcium level yields *downstream processes* (activation of the calmodulin/calcineurin pathway, dephosphorylation of the nuclear factor of activated T cells (NFAT), and the NFAT-sensitive promoter) towards the *target transgene expression*.



*embryonic  
differentiation*

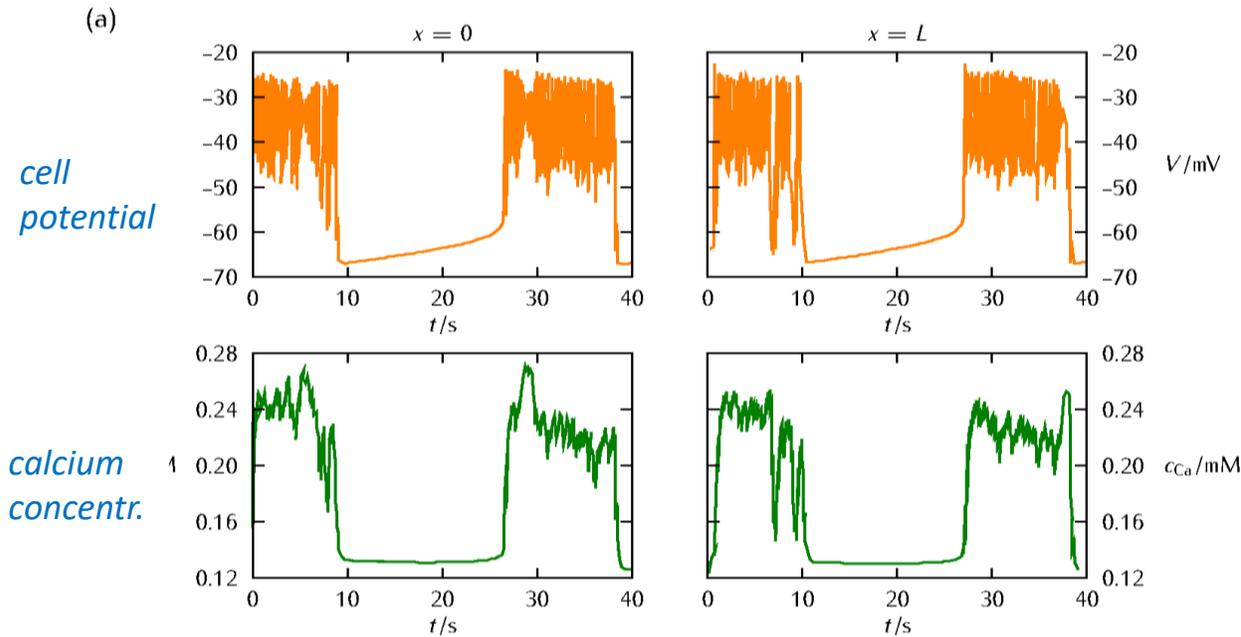
*Membrane potential* (left) and *receptor-ligand* (right) *signaling* in the onset of *embryonic differentiation*. Potassium channels set the polarized cell potential, which keeps voltage-gated calcium channels inactivated, thus avoiding the high calcium levels required for downstream processes to pluripotency. While gene expression is also mediated by intracellular signal transducers and biochemical pathways essential to the expression of differentiation factors, *bioelectricity* may influence also the *timing of pluripotency genes*.

## excitation–transcription coupling in synaptic plasticity



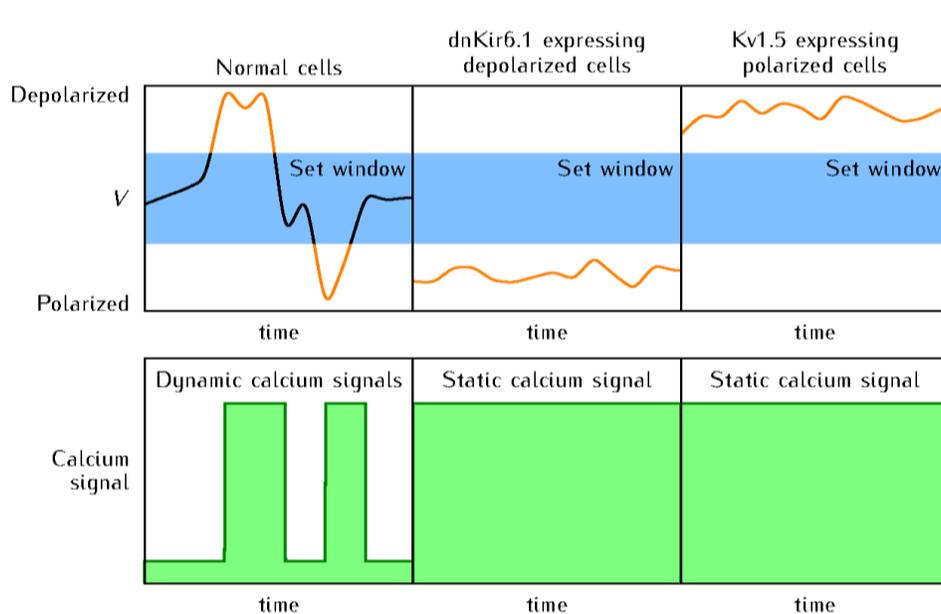
A model of *lasting synaptic plasticity* relies on neuronal activity-induced transcription in the nucleus and transport of translated proteins back to the synapse. The conversion of neuronal excitation to gene expression involves an *excitation–transcription coupling* initiated by membrane depolarization at the synapse and the soma, which drives *Ca<sup>2+</sup> entry via ion channels and activates Ca<sup>2+</sup>-sensitive transcriptional signaling cascades* (red arrows, left). The gene expression yields macromolecules from the nucleus back to the synapse (blue arrow, right) to sustain long-term potentiation.

## experimental coupling between bioelectrical and biochemical ( $\text{Ca}^{2+}$ ) signals

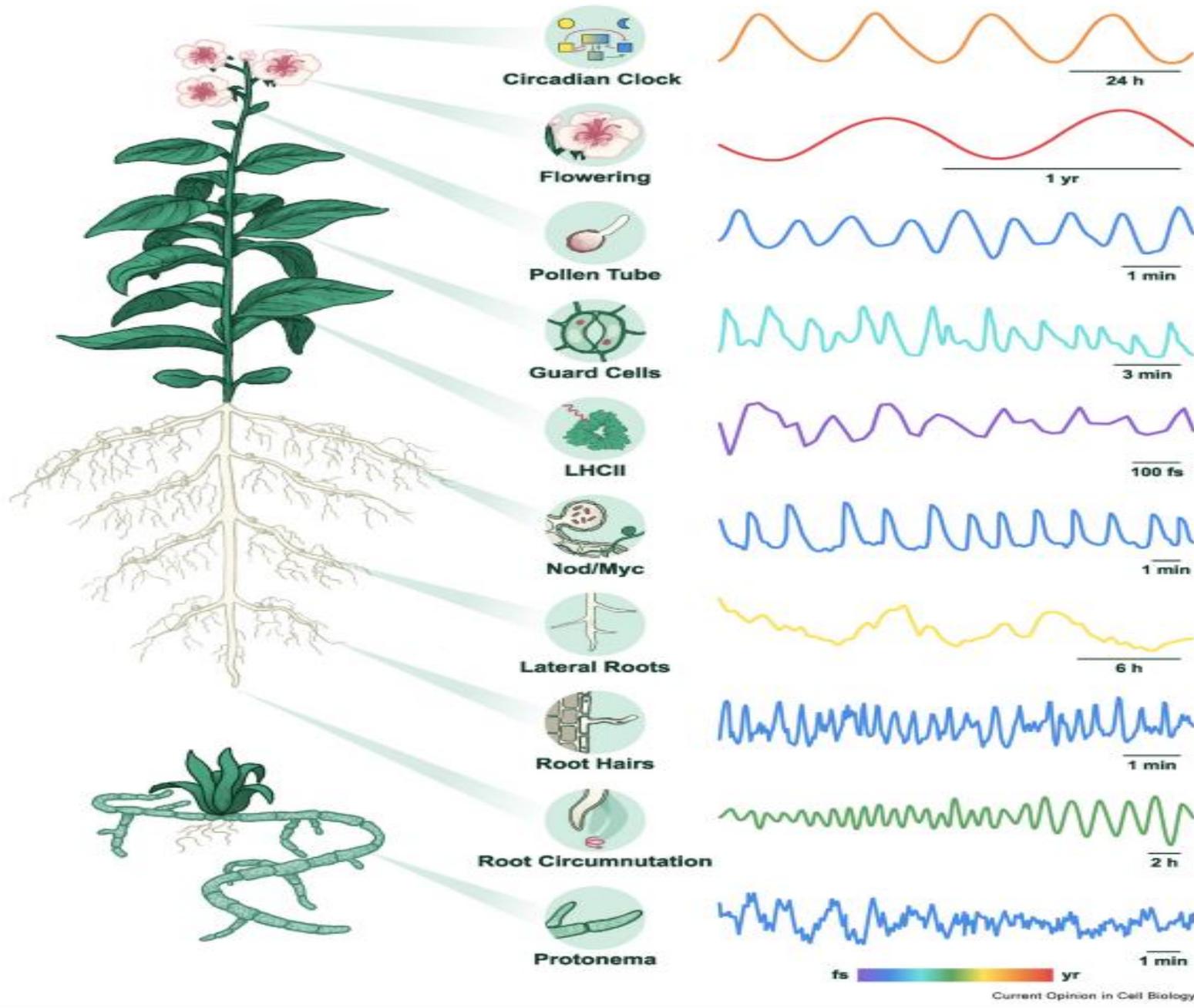


(a) The propagation of a bioelectrical excitation wave in multicellular *pancreatic islets*. The activity time delay for the first ( $x = 0$ ) and last ( $x = L$ ) cells of a linear chain is shown. Note *correlation* between the *bi-stable* time traces of the *cell potential*  $V$  and the intracellular *calcium concentration*  $cCa$ .

(b) *Cell potentials* and *cytoplasmic calcium levels* are interrelated in *Xenopus embryogenesis*. Normally developing cells show a *potential window*. Polarization and depolarization phenomena associated with the expression of two potassium channels (inward-rectifying pore-mutant dnKir6.1 and Kv1.5) cause elevations of calcium levels that fade over developmental time.



These examples suggest that *ion channels*, *cell potentials*, and *signaling ions levels* can be *coupled* in *oscillatory* and *bi-stable memories*.



*Plants* also generate electric potential and  $\text{Ca}^{2+}$  level *oscillations* in many spatio-temporal scales of biological organization. Also, *calcium-binding proteins* influence key *transcriptional processes* here.

*Ion channels* are involved in most *oscillatory regulations*, as shown by optogenetic techniques, which allow direct manipulation of membrane potential and hypothesis testing.

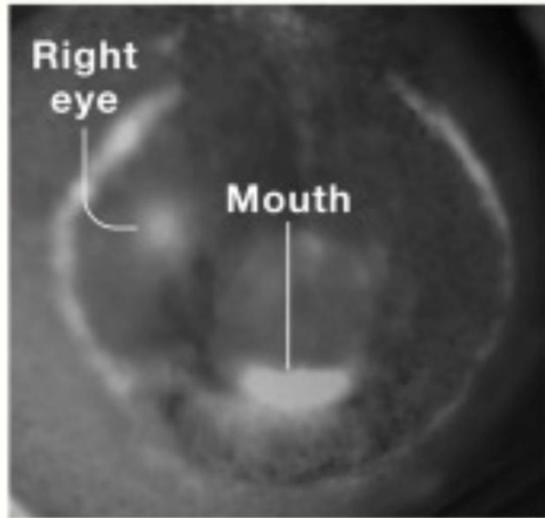
### 3.d Experimental systems and facts

Are *ion channels*, *cell potentials*, and *signaling ions/molecules levels coupled* in *oscillatory* and *bi-stable memories*?

- *Homeostatic channel regulation in neurons*
- *Cell cycle*
- *Electro-genetic circuits*
- *Embryonic differentiation*
- *Excitation-transcription coupling in synaptic plasticity*
- *Developmental processes*
- *Calcium levels in animals and plants*

suggest that *bioelectricity* can influence *protein expression* through a *small number* of *counteracting voltage-gated channels* that act together with other transporters such as *ligand-gated channels* in complex downstream processes. Spatio-temporal distributions of multicellular potentials are then related with gene expression patterns.

### 3.c Multiscale bioelectricity: patterns of cell potentials as a real-time, high scale spatio-temporal integration (Michael Levin)



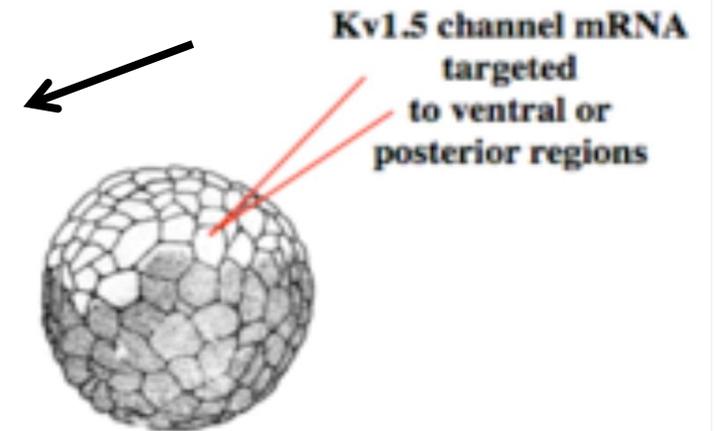
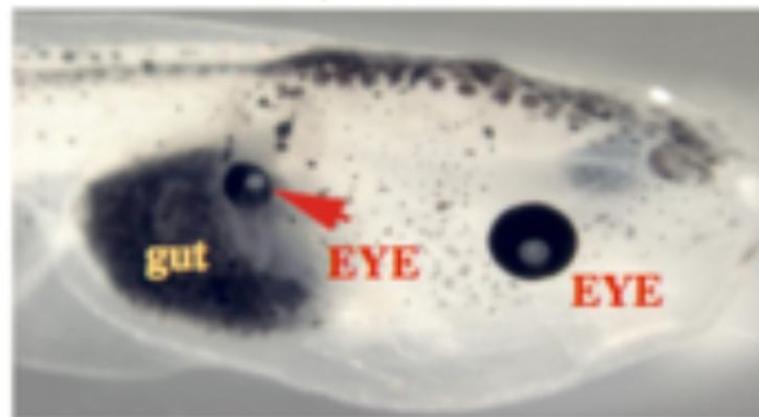
*Voltage-sensitive fluorescent dyes* non-invasively reveal bioelectric patterns.

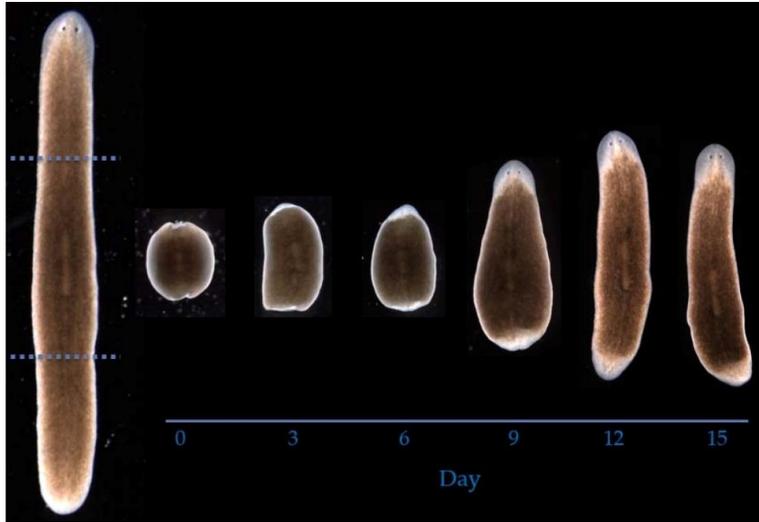
*Multicellular potentials* act as functionally *instructive patterns*.

The *bioelectrical face*: prior to organ generation, fluorescence dyes show hyperpolarized regions where the face organs (eye, mouth) will develop.

Hyperpolarized  Depolarized

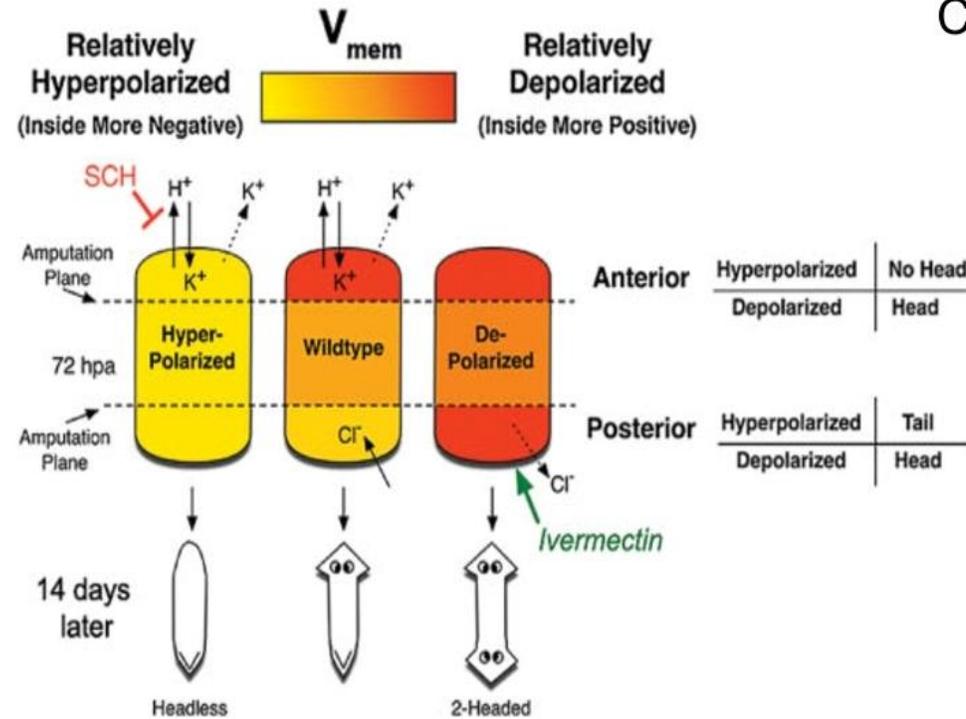
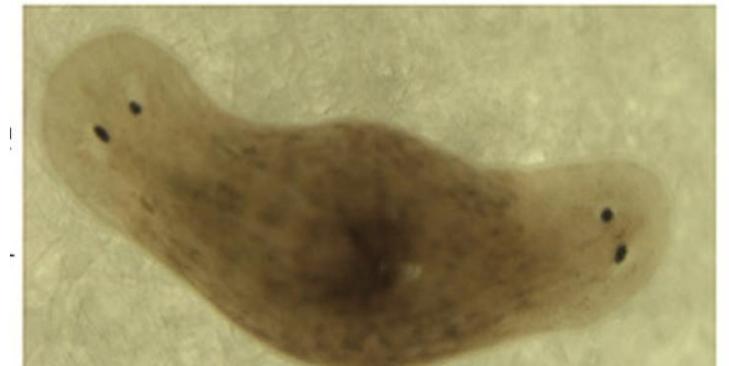
Experimental tools can *read* and *write* the *bioelectric patterns* of non-neural tissues using molecular-genetics, pharmacological, and optogenetical techniques to open and close ion channels and gap junctions.





*Planaria* can regenerate their body upon amputation.

By controlling the *multicellular potential map*, normal, headless, and double-head planaria can be obtained.

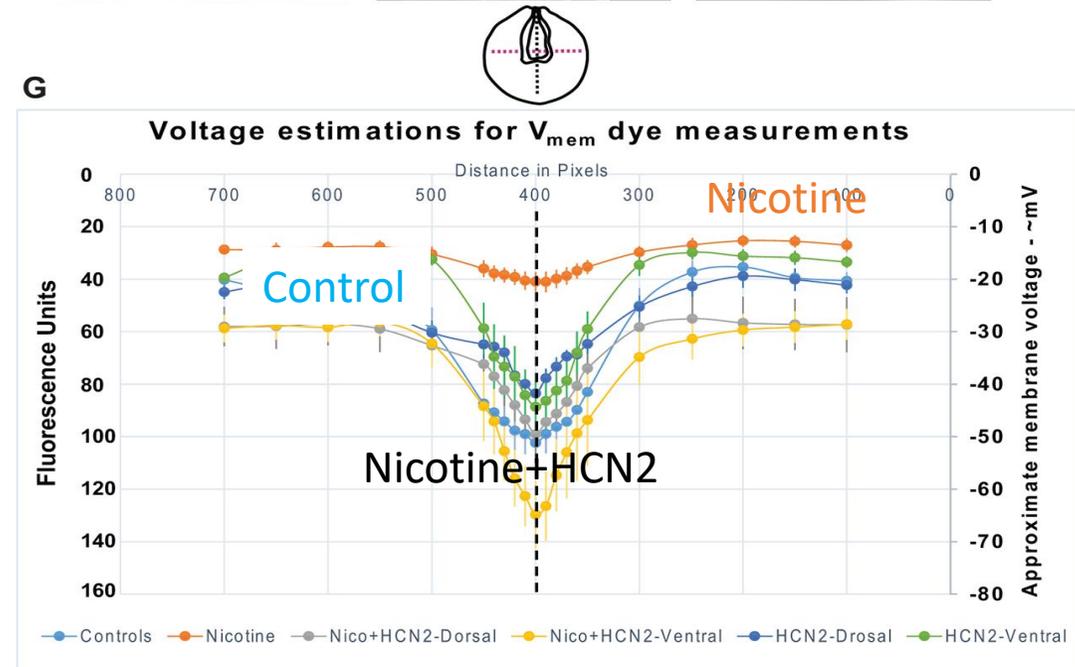
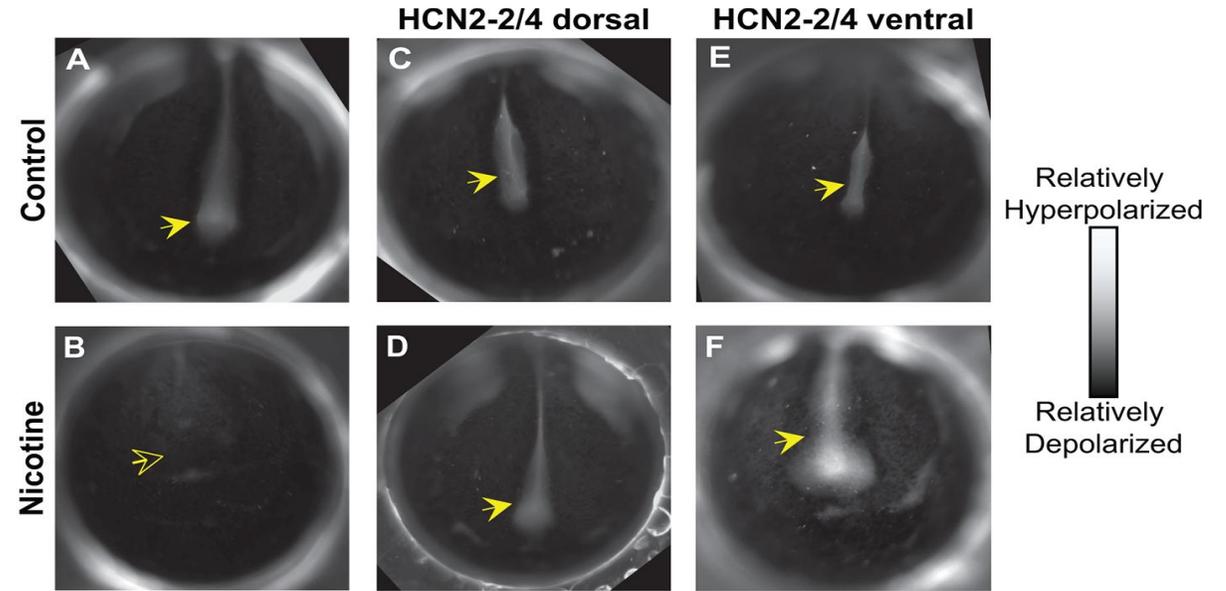


Co

*Pattern completion* by *bioelectrical modules* may allow generating the entire anatomy from a small piece. The bioelectric network stores a pattern memory that controls individual cells to restore the whole organism.

*Embryonic* exposure to teratogen *nicotine* results in *brain defects*, apparently by *disrupting* the *multicellular potential* endogenous pre-pattern.

*Upregulation* of an *ion channel* (*HCN2*) can recover the potential pre-pattern *at distance*, acting to reverse profound defects and *rescue* brain anatomy and learning.



*Nat. Commun.* 2018  
 10.1038/s41467-018-03334-5  
*Front. in Cell. Neurosci.* 2020  
 10.3389/fncel.2020.00136  
*iScience* 2023  
 10.1016/j.isci.2023.108398

## 4. Bioelectrical model assumptions and equations

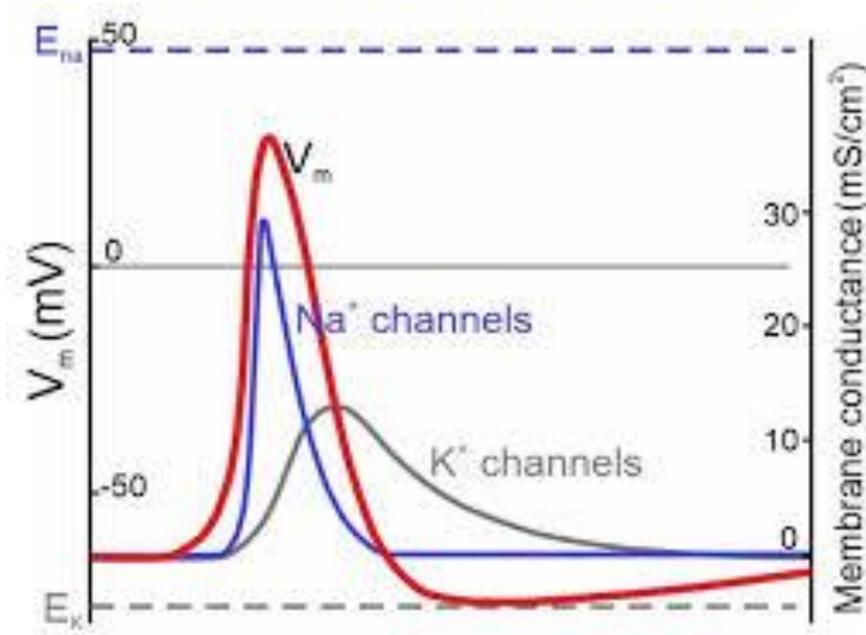
Sci. Rep. 2016  
10.1038/srep35201  
J. Phys. Chem. Lett. 2020  
10.1021/acs.jpcclett.0c00641

*Crucial* points for a simple *bioelectrical* model:

1) many *ion channels* are *voltage-sensitive* and operate over a *wide voltage window*: channels can *polarize* or *depolarize* the cell depending on the particular cell state and the environmental conditions;

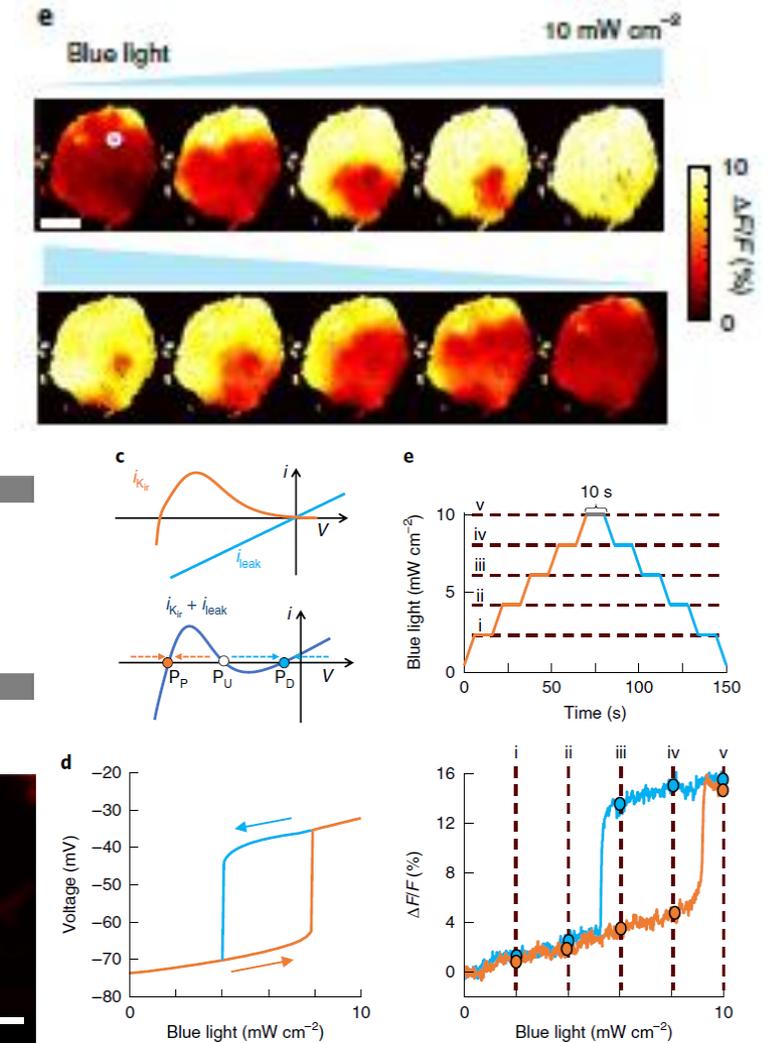
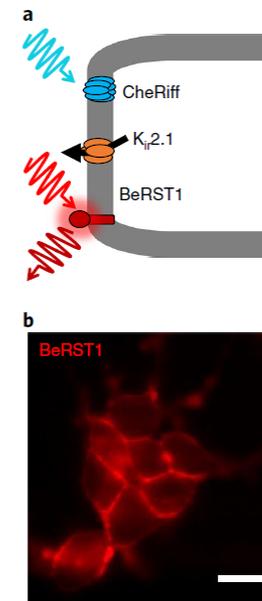
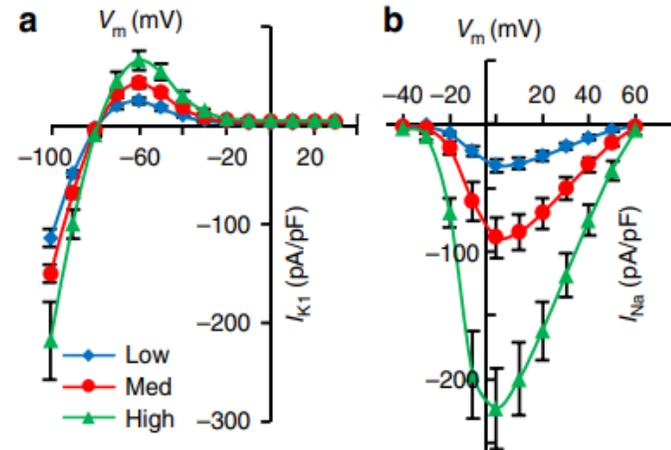
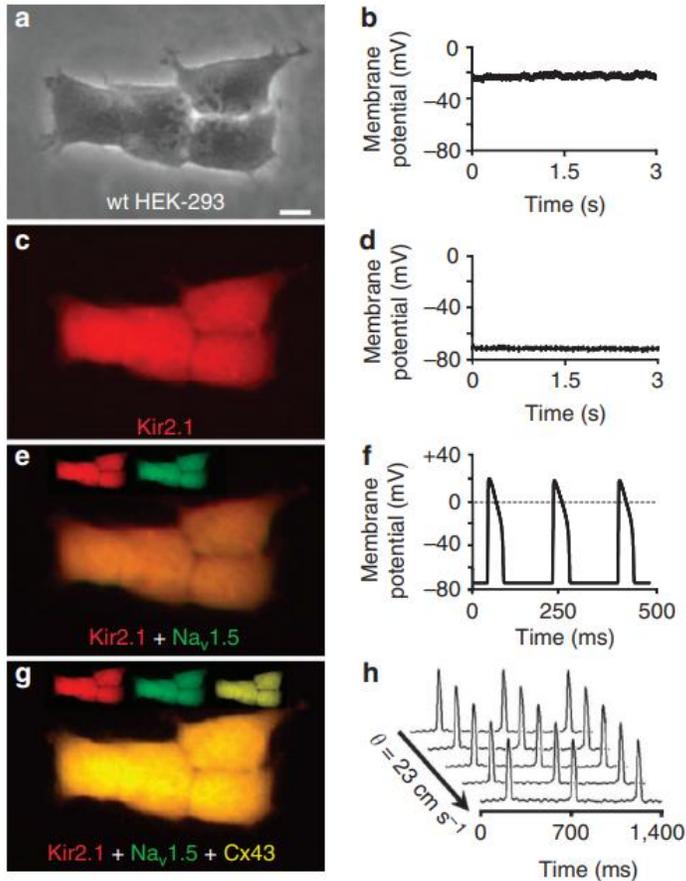
2) the voltage-gated conductances of a *small number* of *counteracting channels* can regulate the polarized (*pol*) and depolarized (*dep*) cell states.

In a different but related problem, the action potential concerns the combined action of *sodium* and *potassium* channels in *depolarization* and *polarization* phenomena;



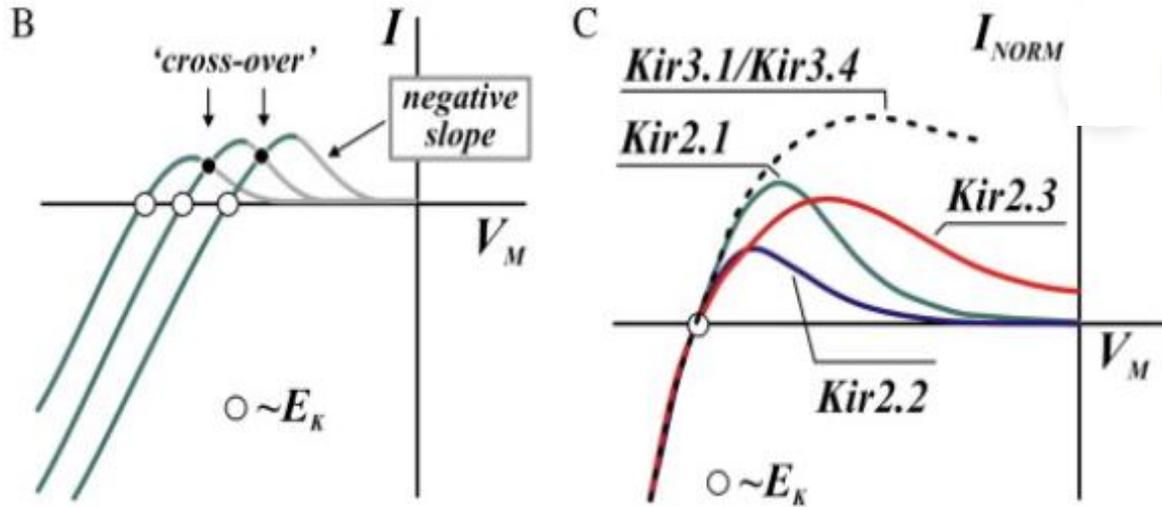
Biosynthetic *excitable tissues* can be designed from non-excitable cells using a *small number* of channels and intercellular junctions, with the formation of *domains* of *bi-stable cell potentials*;

Nat. Commun. 2011, 2016  
 10.1038/ncomms1302  
 10.1038/ncomms13132  
 Nat. Phys. 2020  
 10.1038/s41567-019-0765-4

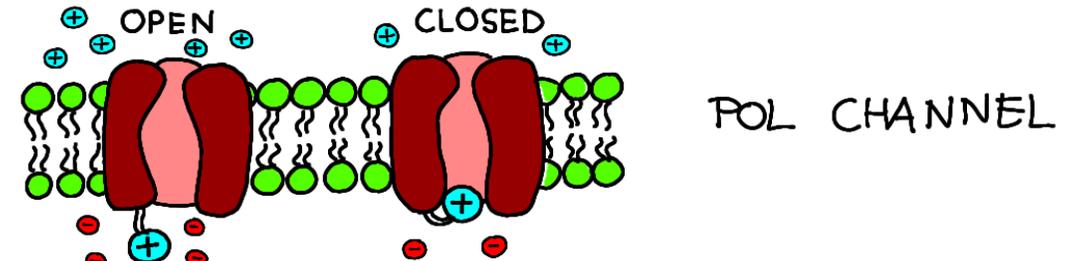


# 4a Single-cell bioelectrical model

J. Mol. Cell. Cardiol. 2009  
 10.1016/j.yjmcc.2009.08.013  
 J. Phys. Chem. B 2014  
 10.1021/jp508304h



*K<sup>+</sup> channel  
 I-V curves*



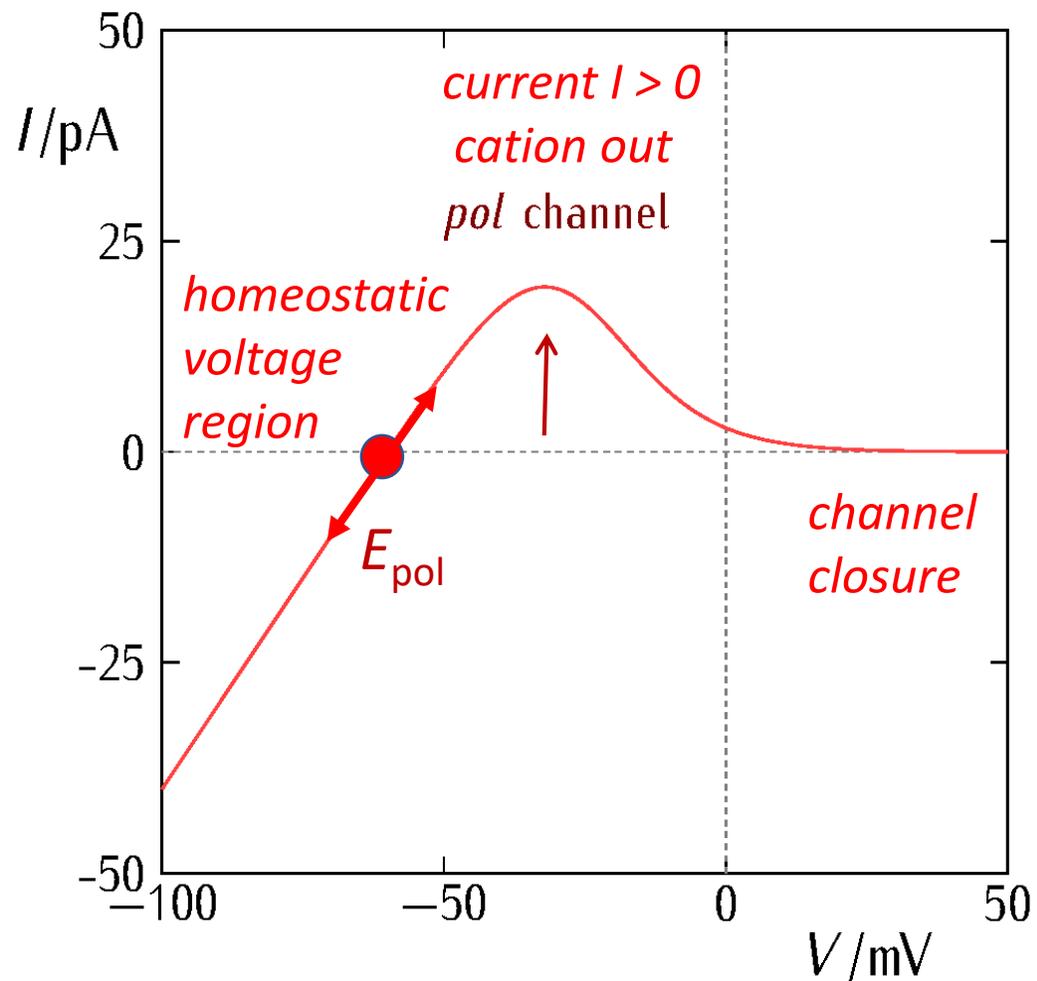
$$I_{pol} = G_{pol}^* \cdot \frac{1}{1 + e^{\frac{z(V - V_{th})}{V_T}}} (V - E_{pol})$$

MAXIMUM CONDUCTANCE  $G_{pol}^*$       NERNST POTENTIAL (CREATED BY PUMPS)  $E_{pol}$

$P_{open}$

$z$  : EFFECTIVE GATE CHARGE  
 $V_{th}$  : THRESHOLD (V FOR  $P_{open} = 1/2$ )  
 $V_T = \frac{kT}{e}$  ← ELEMENTARY CHARGE

In the model, a *generic pol* conductance simulates a *diverse family* of channels acting to *polarize* the cell.



*context-sensitive:*

channel can repolarize the cell by correcting a *small* depolarization (*homeostatic* region, disruptive event *suppressed*)

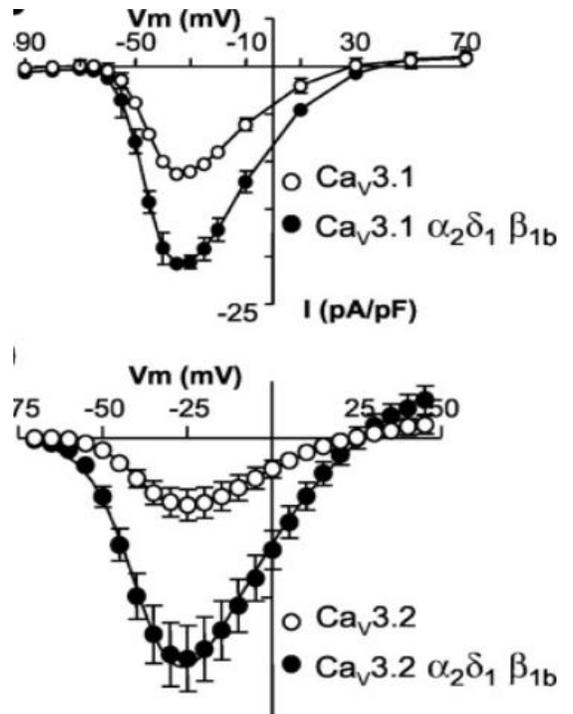
but

can permit a *large* depolarization (channel *closure*), thus allowing a

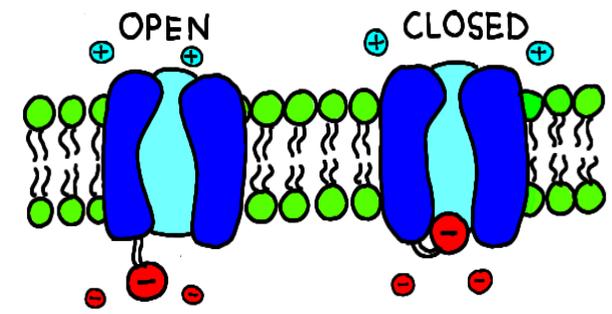
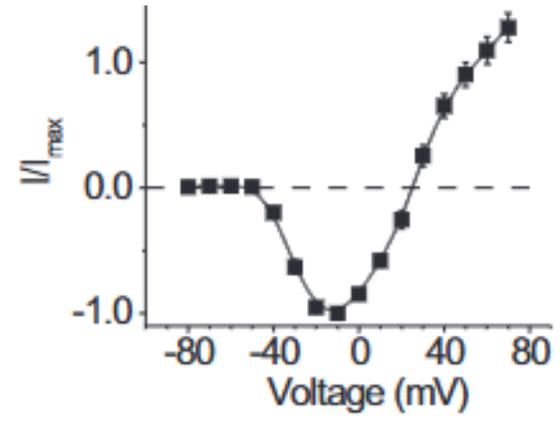
*bioelectrical regionalization*

PNAS 2012  
 10.1073/pnas.1115575109  
 J. Biol. Chem. 2004  
 10.1074/jbc.M313450200  
 J. Phys. Chem. B 2014  
 10.1021/jp508304h

*Ca<sup>2+</sup> channel I-V curves*



*Na<sup>+</sup> channel I-V curve*



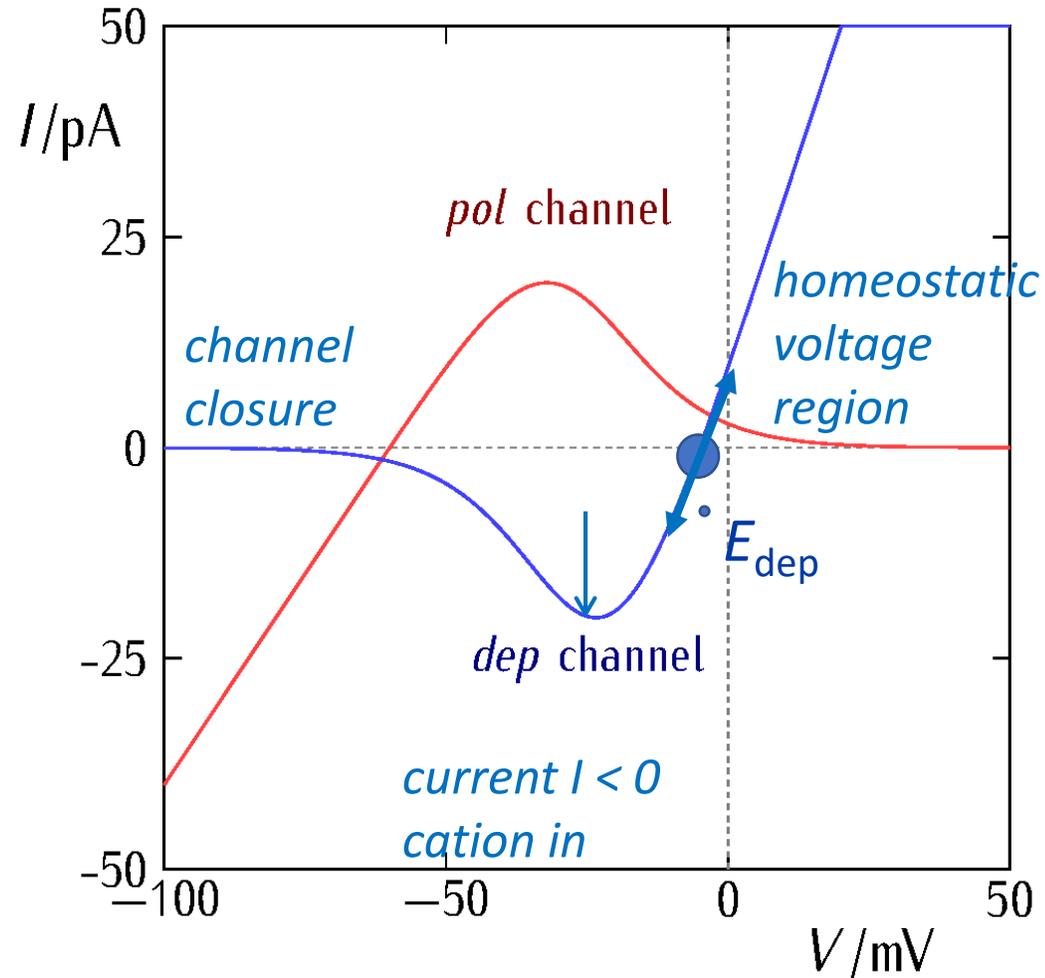
DEP CHANNEL

$$I_{dep} = G_{dep}^* \cdot \frac{1}{1 + e^{\frac{z(V - V_{th})}{V_T}}} (V - E_{dep})$$

MAXIMUM CONDUCTANCE  $G_{dep}^*$        $\frac{1}{1 + e^{\frac{z(V - V_{th})}{V_T}}}$       NERNST POTENTIAL (CREATED BY PUMPS)  $(V - E_{dep})$   
 $P_{open}$

$z$  : EFFECTIVE GATE CHARGE ( $z < 0$ )  
 $V_{th}$  : THRESHOLD (V FOR  $P_{open} = 1/2$ )  
 $V_T = \frac{kT}{e}$  ← ELEMENTARY CHARGE

In the model, a *generic dep* conductance simulates a *diverse family* of channels acting to *depolarize* the cell.



*context-sensitive:*

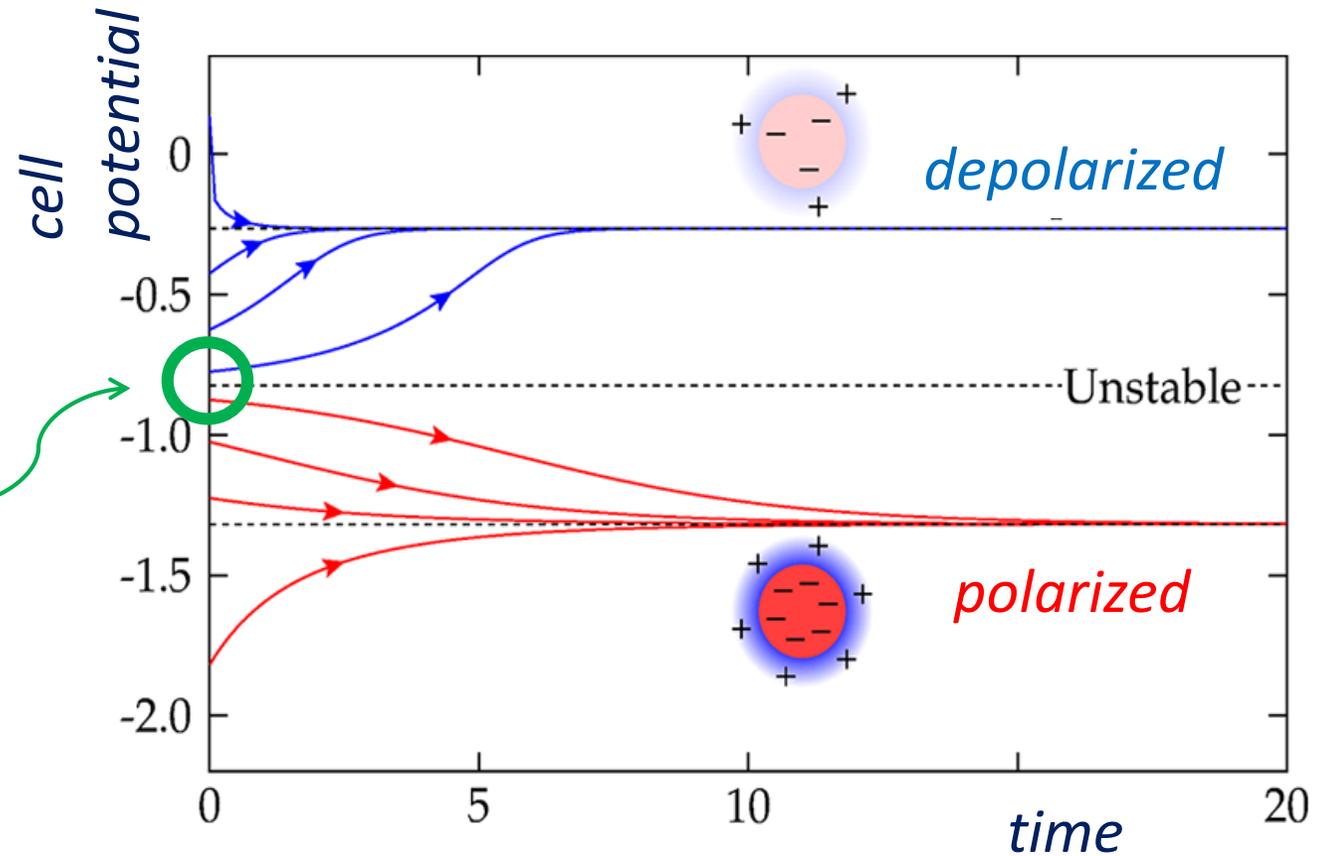
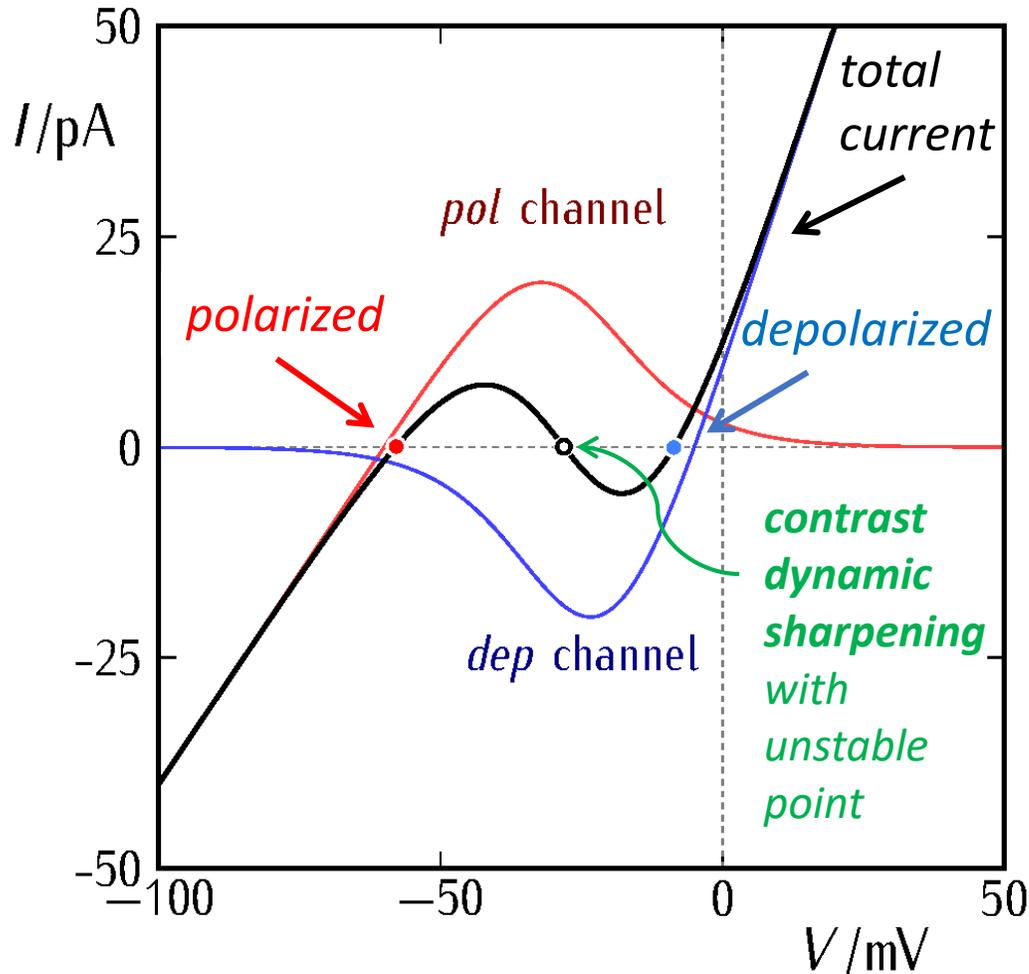
channel can depolarize by correcting a *small* repolarization (*homeostatic* region, disruptive event *suppressed*)

but

can permit a *large* repolarization (channel *closure*), thus allowing a

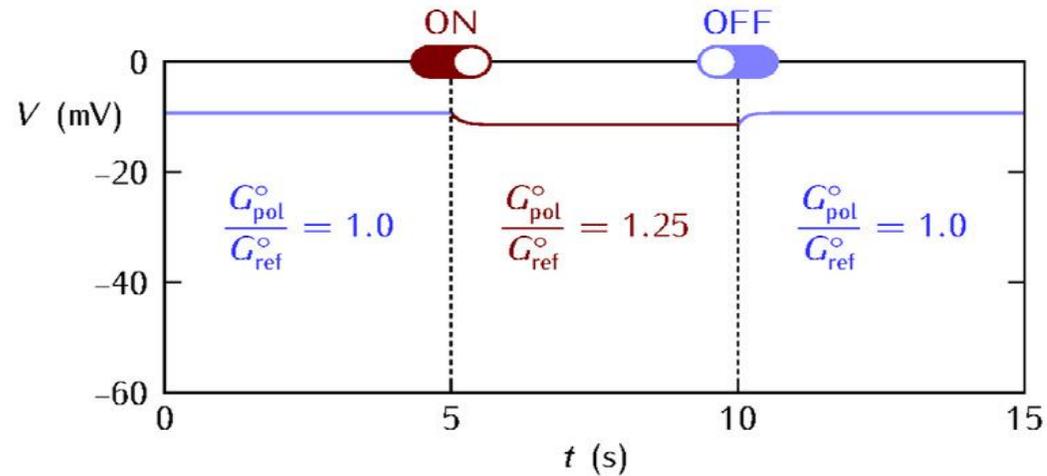
*bioelectrical regionalization*

**Contrast dynamic sharpening:** the *combination* of the two channels over a *wide voltage window* may *polarize* or *depolarize* the cell depending on the particular cell state and the environmental conditions. The *combined action* of the two generic channels *pol/dep* gives a cell potential *bi-stability* that allows *contrast sharpening* in *bioelectrical regionalization*.

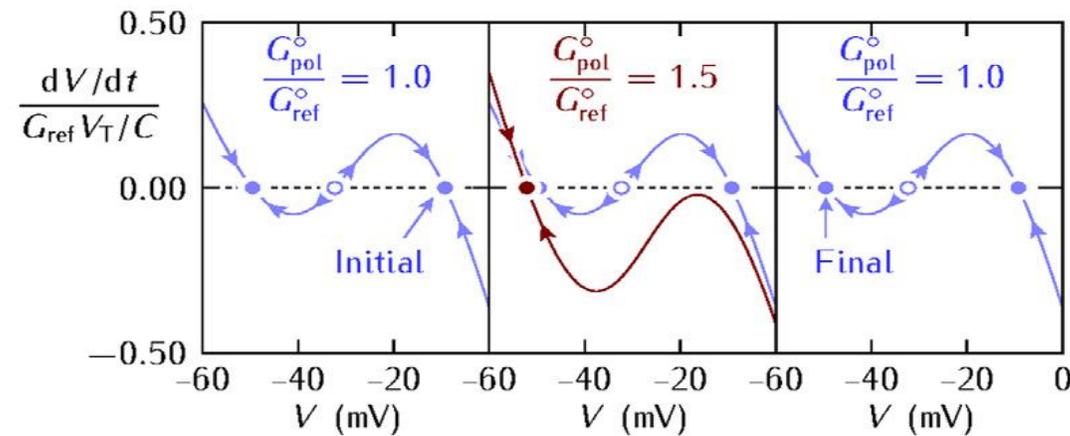
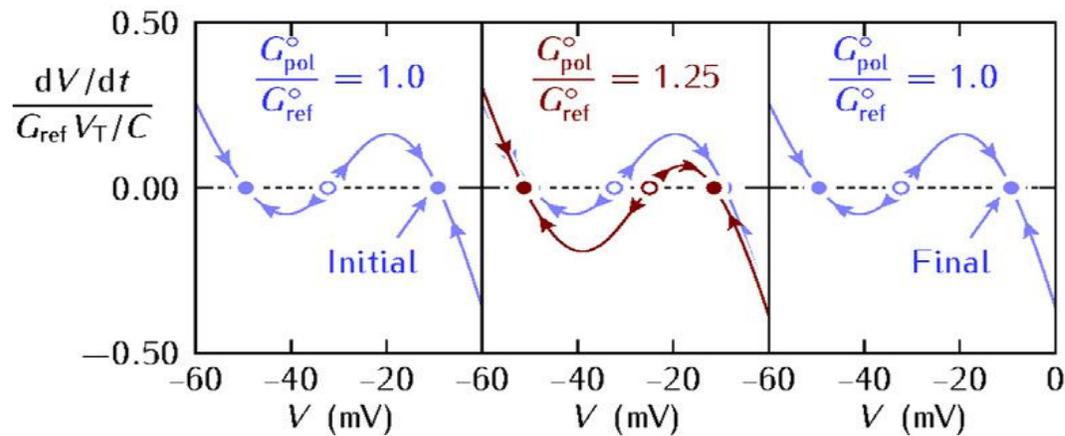
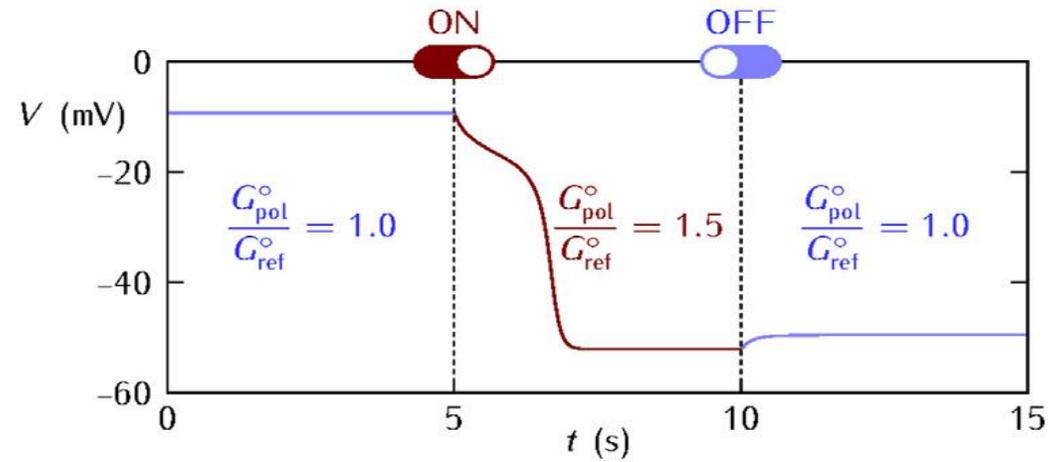


Channels may support *stable single-cell memories* based on membrane potentials. A *perturbation* or *disruptive* event (e.g. the over-expression or blocking of a specific channel) changes the state of a cell of capacitance  $C$  *only* if sufficiently *strong*. Instructive changes could be established by *transient, limited actions*.

(a) Weakly perturbed

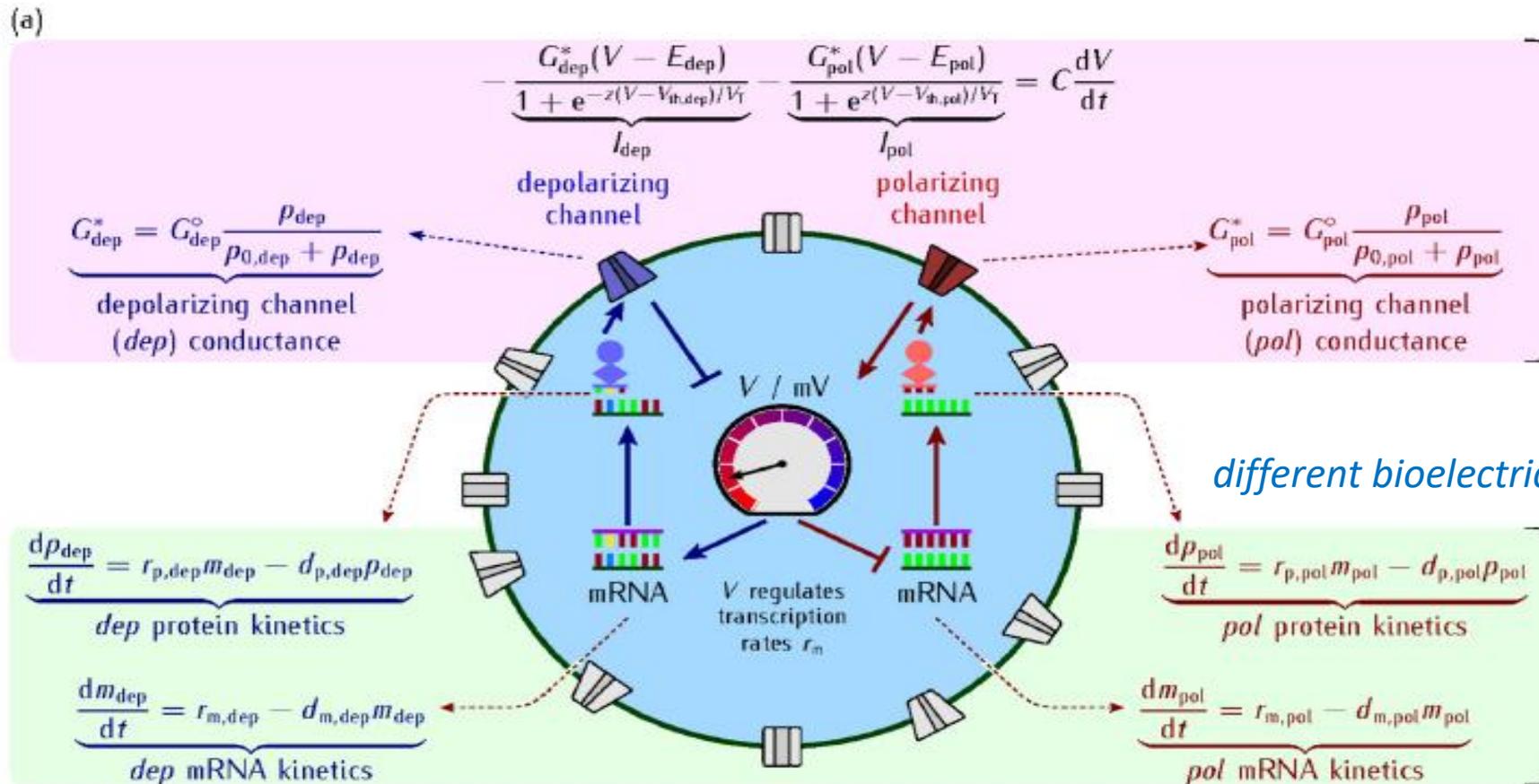


(b) Strongly perturbed



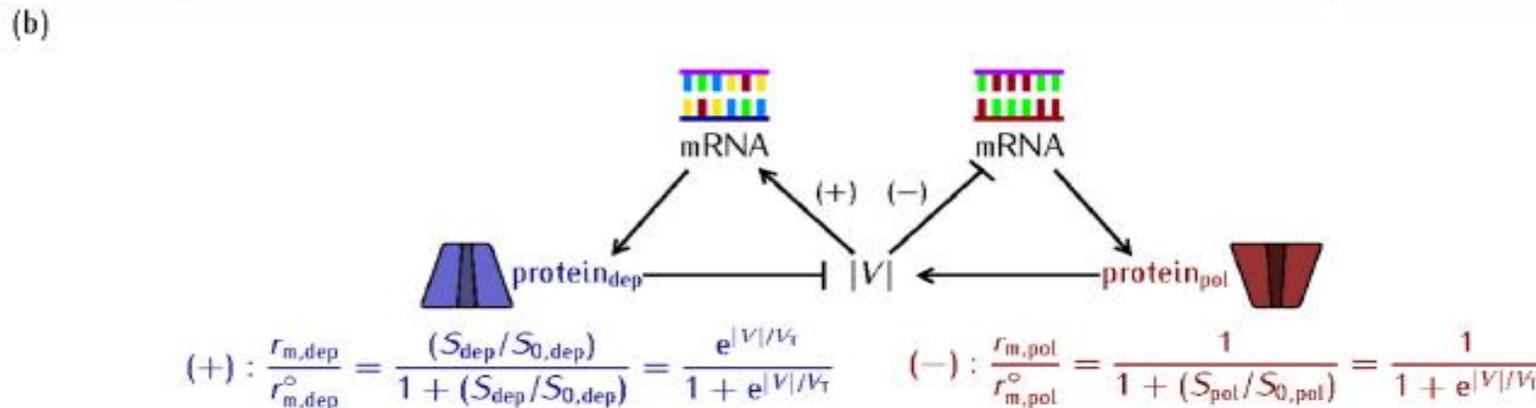
$$C(dV/dt) = -I_{pol} - I_{dep}$$





*Bioelectrical network*  
 (ion channel dynamics:  
*seconds to minutes*)

*Transcriptional network*  
 (ion channels expression:  
*hours to days*)

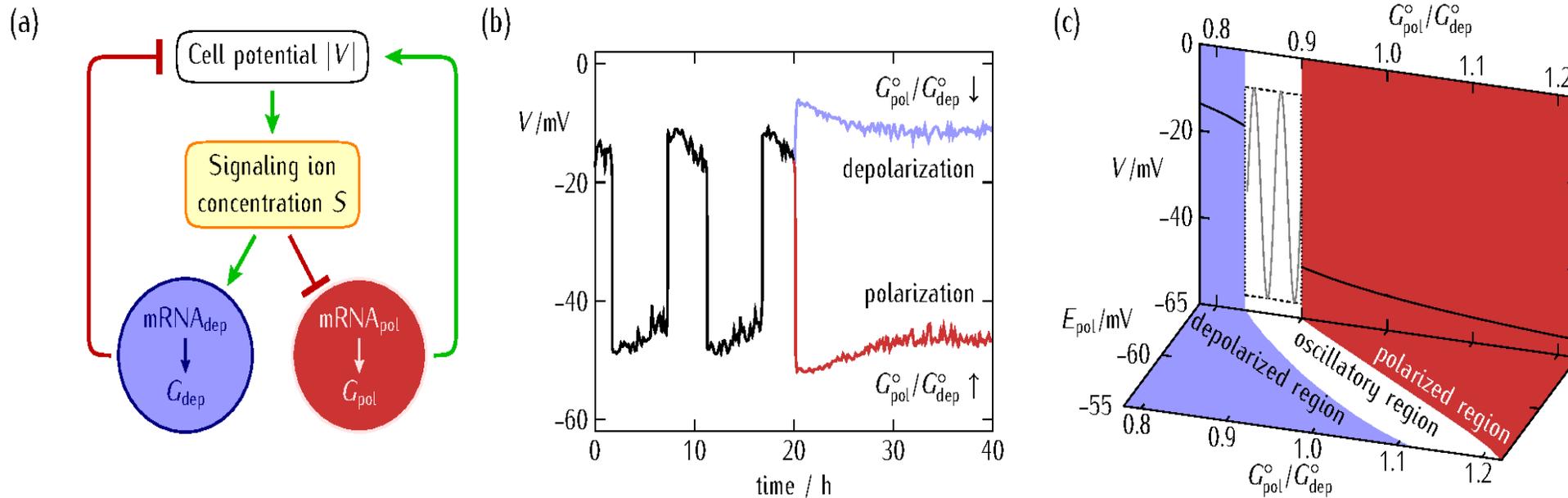


The *cell membrane potential V* can influence *transcription via second-messengers*, e.g. calcium: its cytoplasm concentration *S* may show a *positive (dep channel here)* or *negative (pol channel here)* regulation on *V*.

In this bioelectrical model:

- the transcription and translation of the *dep* and *pol* channel proteins is described in terms of the *mRNA* ( $m_{dep}$  and  $m_{pol}$ ) and *protein* ( $p_{dep}$  and  $p_{pol}$ ) *concentrations*;
- the *rate constants*  $r_{m,k}$  and  $r_{p,k}$  ( $k = dep, pol$ ) regulate the mRNA transcription and protein translation processes, respectively, which occur with the respective *degradations rates*  $d_{m,k}$  and  $d_{p,k}$  ( $k = dep, pol$ );
- the *conductances*  $G_k^* = G_k^0 p_k / (p_{0,k} + p_k)$  ( $k = dep, pol$ ) follow a *Hill kinetics* with the respective protein concentrations  $p_k$ , where  $G_k^0$  is the *maximum conductance* and  $p_{0,k}$  is the *reference protein concentration* which gives the half-maximum conductance. This kinetic saturation of the *dep* and *pol* channel conductances reflects the finite values of the transcription and translation rates and may also incorporate the limits imposed by the protein trafficking to and insertion in the cell membrane. The action of specific blockers can also be introduced by decreasing the effective values of the maximum conductance  $G_k^0$ ; and
- the *coupling* between transcription and bioelectricity results in *cell potential V-dependent mRNA transcription rates*  $r_{m,k}(V)$  where  $r_{m,k}^0$  are the reference values ( $k = dep, pol$ ). Thus,  $V$  modulates the protein concentrations and the resulting channel conductances through the transcription while the channel conductances modulate  $V$  through the post-translational bioelectricity. Note that this coupling should be *system specific*.

## the model allows bi-stable and oscillatory single-cell cell potentials



**important:** change in the *external* or *endogenous conditions*  $\rightarrow$  change in *bioelectric-genetic coupling*  $\rightarrow$  change in the *single-cell configuration space*!

(a) The *cell potential*  $V$  characterizing the *pol* and *dep* states, regulated by the *bioelectrical* and *transcriptional feedback*, for the case of a *signaling ion* of *concentration*  $S$ . (b) The coupling between bioelectricity and transcription leads to *pol* and *dep stable* and *oscillatory* cell potentials, modulated by the maximum conductances ratio and the equilibrium potentials. Here, the channel protein regulation is *negative* for the *pol* channel and *positive* for the *dep* channel. (c) The conductance ratio and equilibrium potential define a *configuration space* for the bioelectrical transitions between the cell states.

## *limitations of the single-cell model*

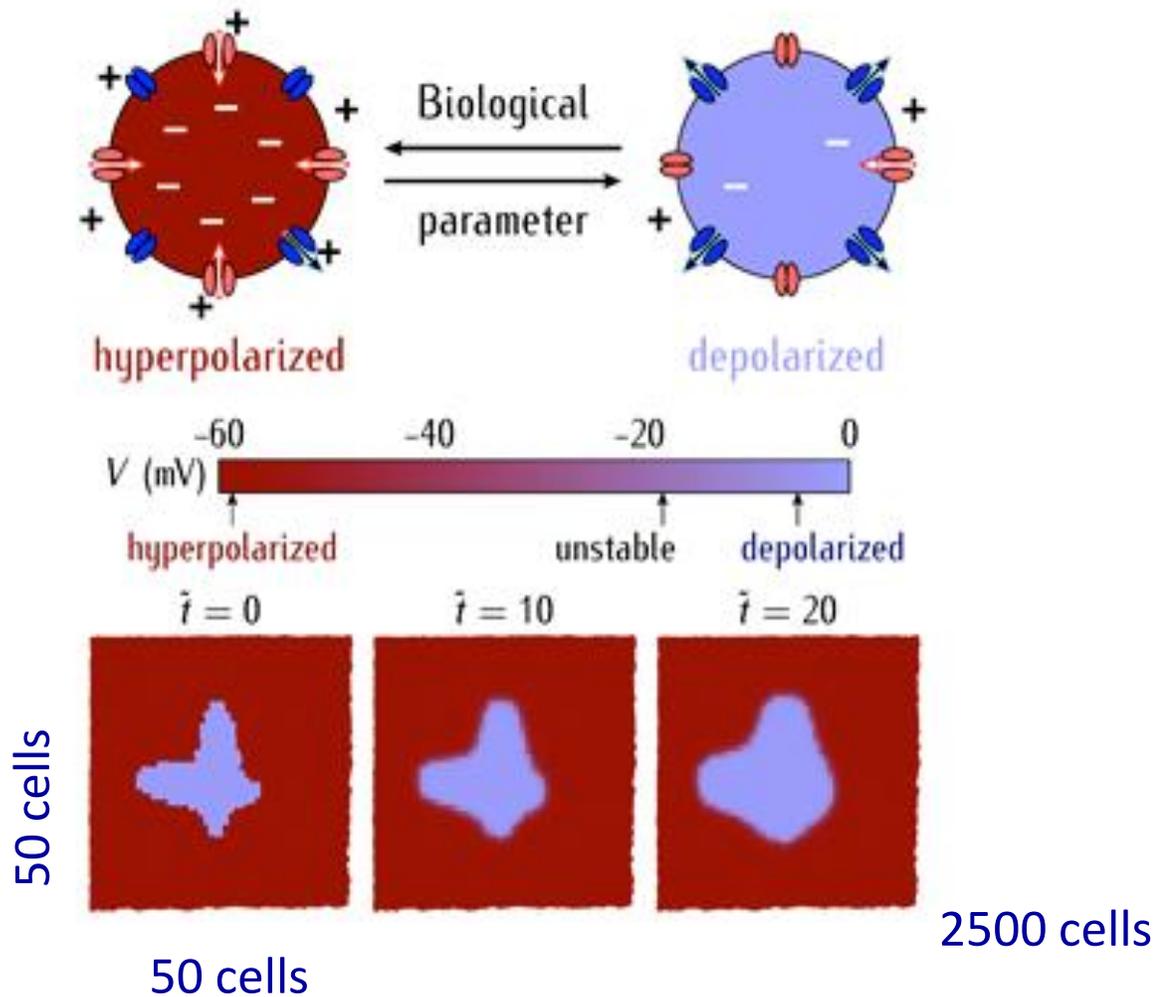
The *bioelectrical-transcriptional coupling* is *system-dependent*: theoretical models may not be universal and depend on the *different channels and mechanisms* relevant to each *experimental case*.

Because channels *polarize* or *depolarize* the cell depending on the particular *cell state* and the *environmental conditions*, externally-induced bioelectrical actions are not only *system-dependent* but also *context-dependent*.

These *serious limitations* should be taken into account when attempting to describe *cell potentials* in particular experimental cases.

## the cell as a bioelectric dynamical system

Low (*depolarized*) and high (*polarized*) cell potential *states* and *transitions* between them.



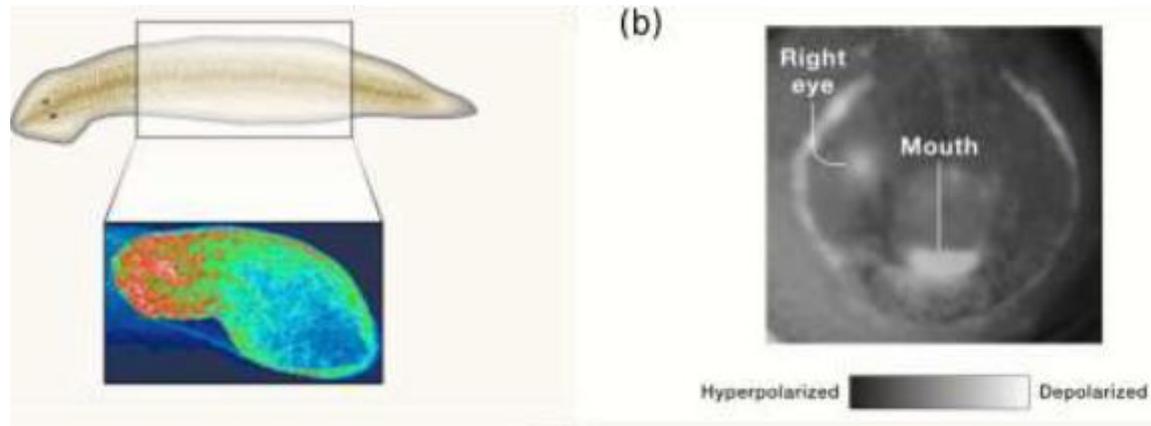
Transitions are induced by changes in:

- 1) *ionic concentrations* (equilibrium potentials) and
- 2) relative values of the *polarizing* and *depolarizing* channels *conductances*.

However, *single-cell* states are coupled at the *multicellular* level. Thus, an *extended* model that includes the *intercellular gap junction* conductance is needed.

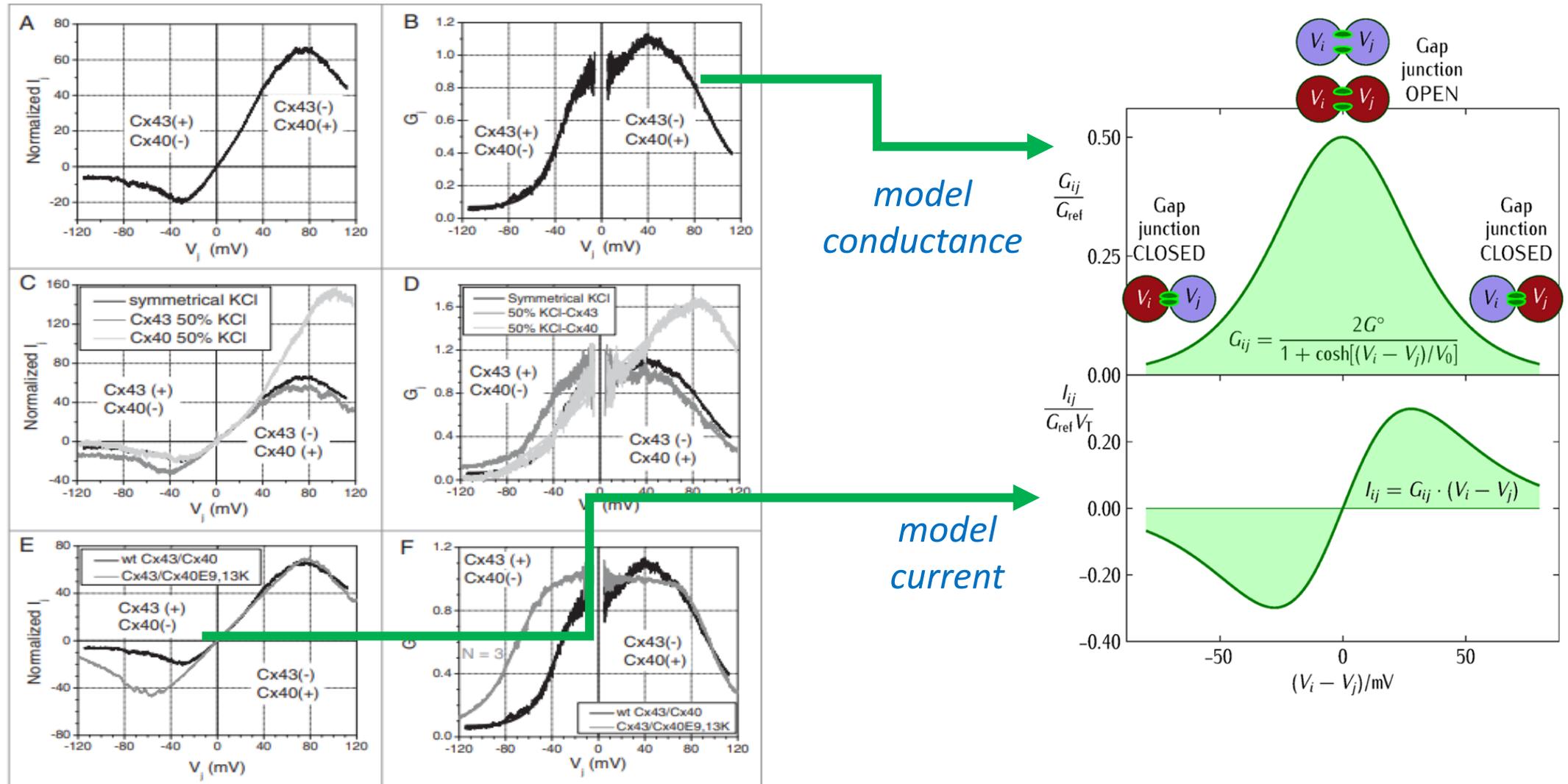
## 4c Extension to the multicellular case: intercellular gap junctions (with José A. Manzanares)

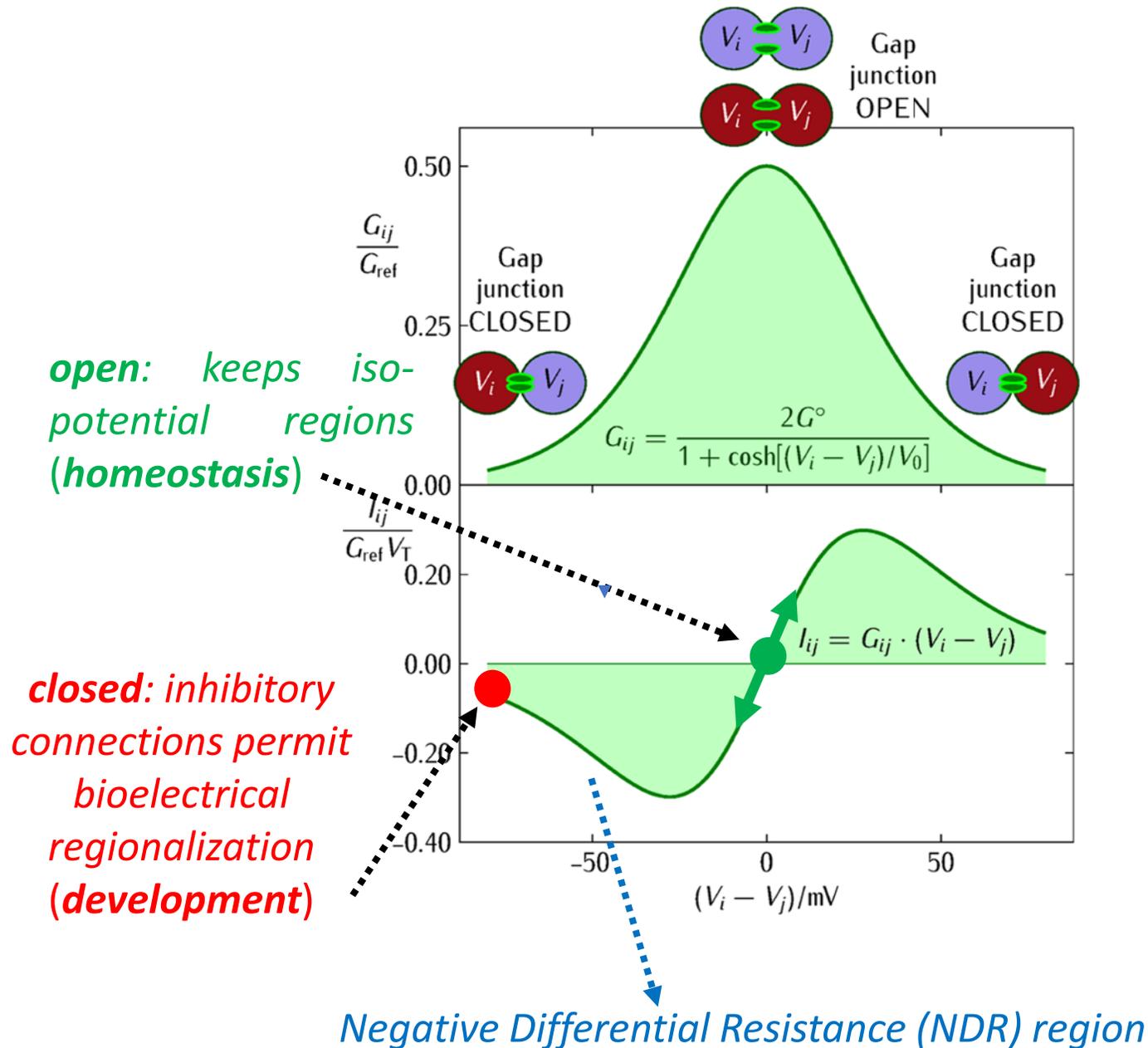
Because of intercellular connectivity, the *single-cell* coupling between bioelectricity and transcription can be extended to the *multicellular level*. In this way, *average potentials* could either *collectively counteract* disruptive local perturbations in cell polarization or *facilitate* morphologically instructive global transitions, as needed in the *different biological stages* shown.



The *bioelectrical regionalization* of the multicellular ensemble in *polarized/depolarized* regions converts *single-cell* states into *multicellular* states. Because cells are sensitive to spatio-temporal bioelectrical patterns, the *different multicellular potential maps* give *distinct downstream gene expression patterns*.

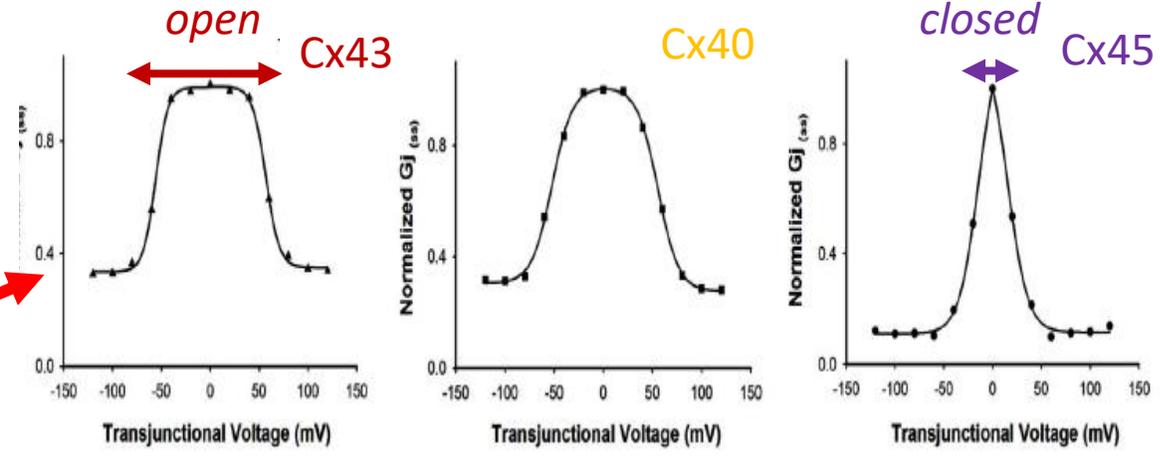
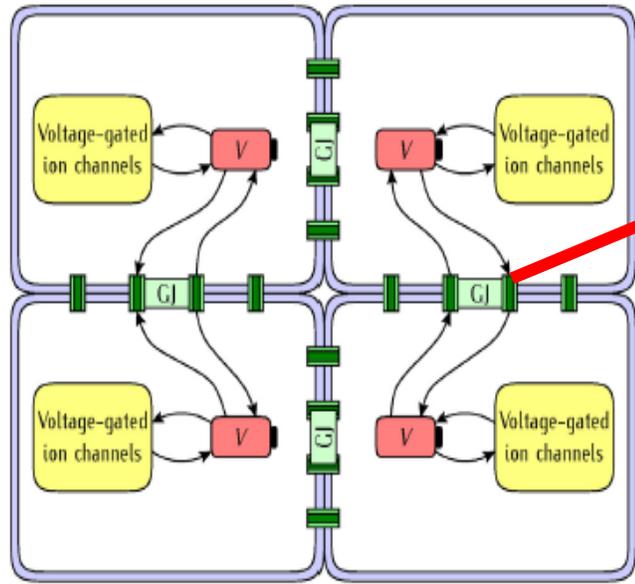
Experimentally, the *junction current* and *conductance*  $G_{ij}$  between two neighboring cells i and j depends on the *intercellular potential difference*  $V_i - V_j$





**Contrast dynamic sharpening:** gap junctions operate over a wide voltage window: they may *polarize* or *depolarize* the cell depending on the bioelectrical neighborhood and environmental conditions, which contributes to the spatio-temporal contrast needed for *bioelectrical regionalization*.

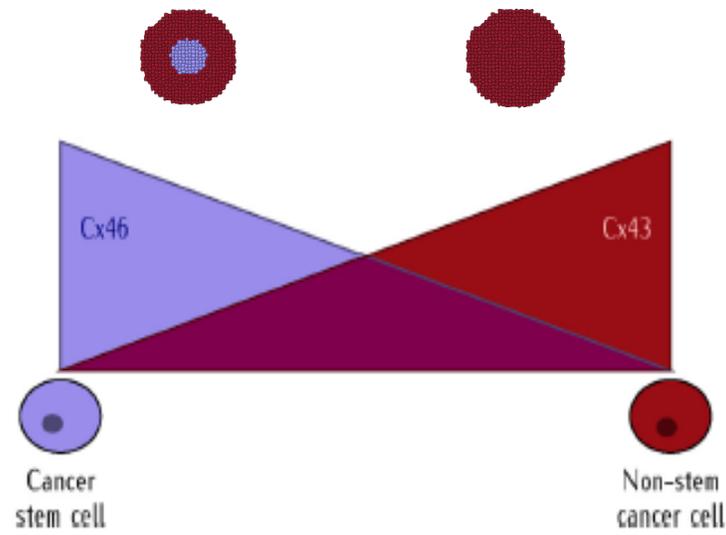
PLoS ONE 2013  
 10.1371/journal.pone.0060506  
 Cell Rep. 2015  
 10.1016/j.celrep.2015.04.021  
 Phys. Rep. 2023  
 10.1016/j.physrep.2022.12.003  
 Cancers 2021  
 10.3390/cancers13215300



*Sensitivity to transjunctional voltage*: closing promotes *bioelectrical regionalization*

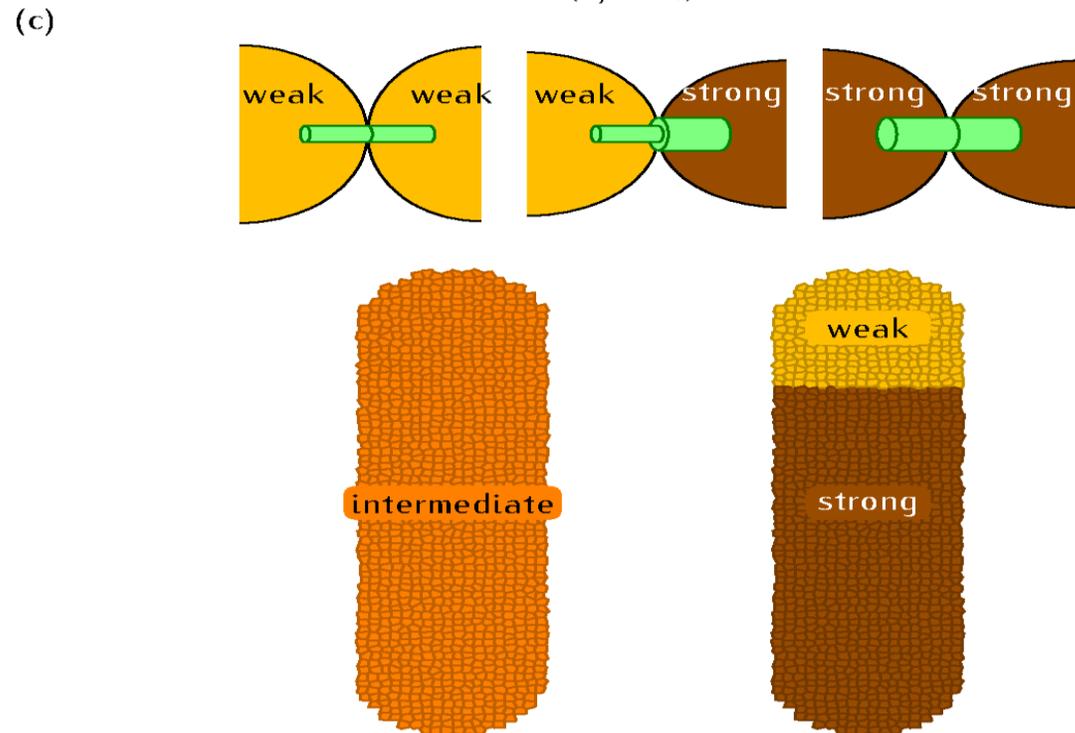
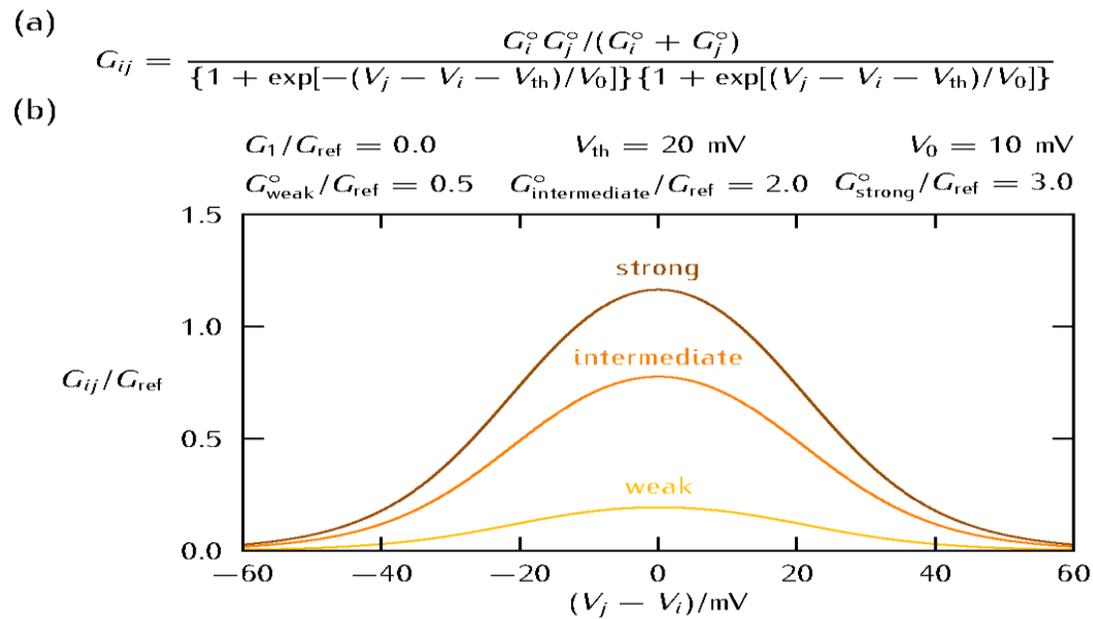
*closed: regionalization?*      *open: no regionalization?*

Single-cell potentials (V) *influence* and *are influenced* by voltage-gated ion channels. In turn, *this feedback* is *coupled by* the *intercellular gap junctions* at the multicellular level.



*Glioblastoma cancer stem cells* may show particular junction proteins. *Different junctions* with *distinct voltage-sensitivities* can perturb multicellular domains.

**Important:** other *biochemical & biomechanical effects* must also be present in this complex problem!



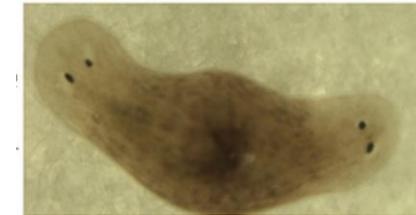
(a) Junction conductances of different connexin proteins obtained with distinct parameters.

(b) The cells  $i$  and  $j$  have maximum conductances that depend on the *individual polarization state* of the cell, which influences the expression of the junction protein.

(c) Homogeneous spatial distribution of junction conductances (*left*) or *regionalizations* into *different modules* (*right*) are possible.

Together, these facts show a *rich network connectivity dynamics*, as suggested by the variety of bioelectric patterns found experimentally.

# bioelectrical configuration space for flatworm morphology depends on junction connectivity

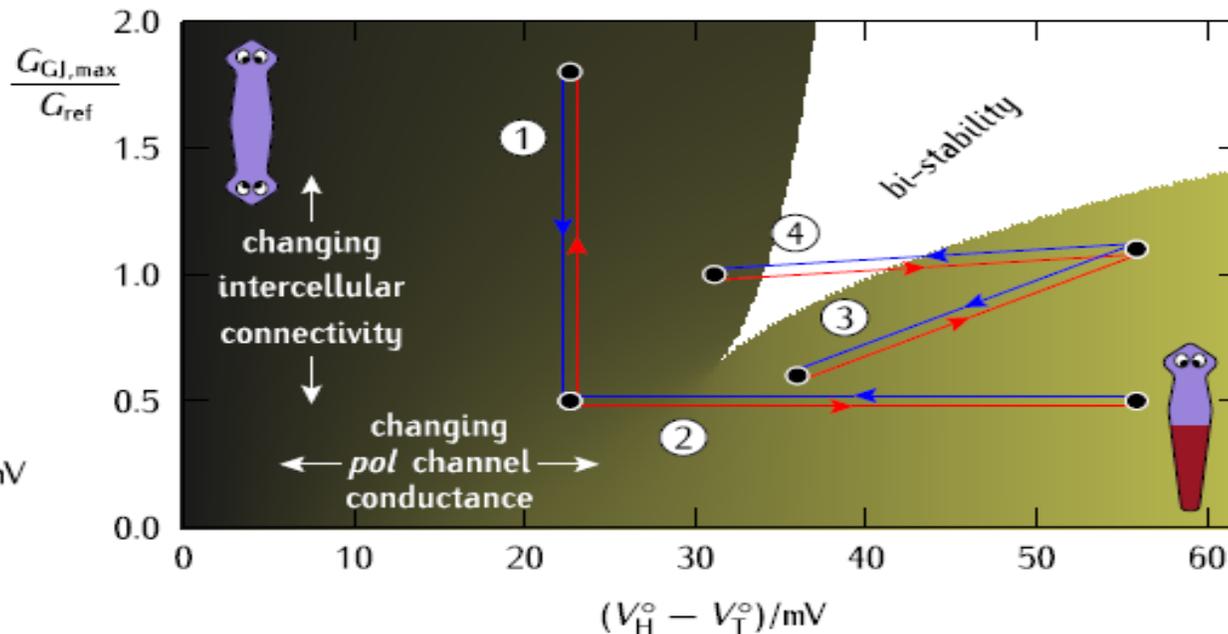
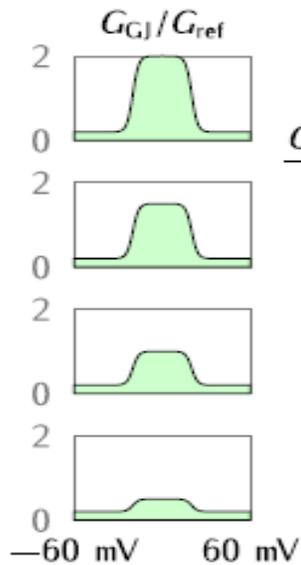
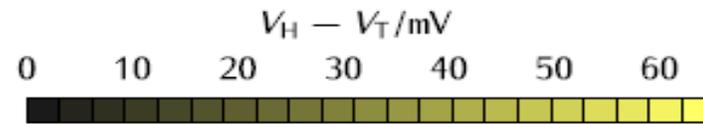


Steady-state solution for isolated cells:

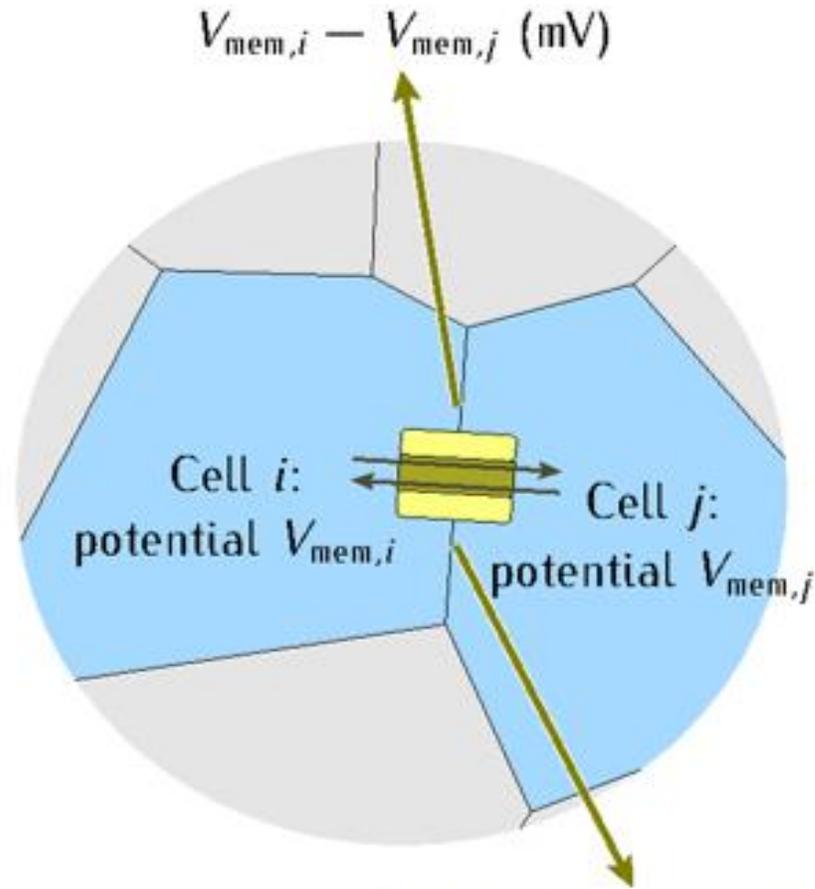
$$V_H^o \equiv \frac{G_{pol,H}^o E_{pol,H} + G_{dep,H}^o E_{dep,H} - I_{pump,H}}{G_{pol,H}^o + G_{dep,H}^o}; \quad V_T^o \equiv \frac{G_{pol,T}^o E_{pol,T} + G_{dep,T}^o E_{dep,T} - I_{pump,T}}{G_{pol,T}^o + G_{dep,T}^o}$$

Steady-state solution for coupled cells:

$$\left[ 1 + \frac{G_{GJ}(V_H - V_T)}{G_{pol,H}^o + G_{dep,H}^o} + \frac{G_{GJ}(V_H - V_T)}{G_{pol,T}^o + G_{dep,T}^o} \right] (V_H - V_T) - (V_H^o - V_T^o) = 0$$



Model equations and resulting *configuration space* for the *head-tail* potential difference  $V_H - V_T$  as a function of the *isolated cell* potential difference (*superindex o*) and the *maximum gap junction conductance*  $G_{GJ,max}/G_{ref}$ . The color gradient indicates the different *monostable solutions*; the central white zone is the *bi-stability region*. The arrows show the trajectories of different processes.



The membrane potential  $V_{\text{mem}}$  of cell  $i$  changes because of the channel currents  $I_{\text{pol}}$  and  $I_{\text{dep}}$  and the *intercellular coupling current*  $I_{ij}$  between cell  $i$  and all neighboring cells  $j$ .

Crucially, the junction allows the *cytoplasm-coupled cells* of a spatial module to form an *isopotential region* that can be *regulated as a whole*, as suggested by the experimental patterns of electrical potentials.

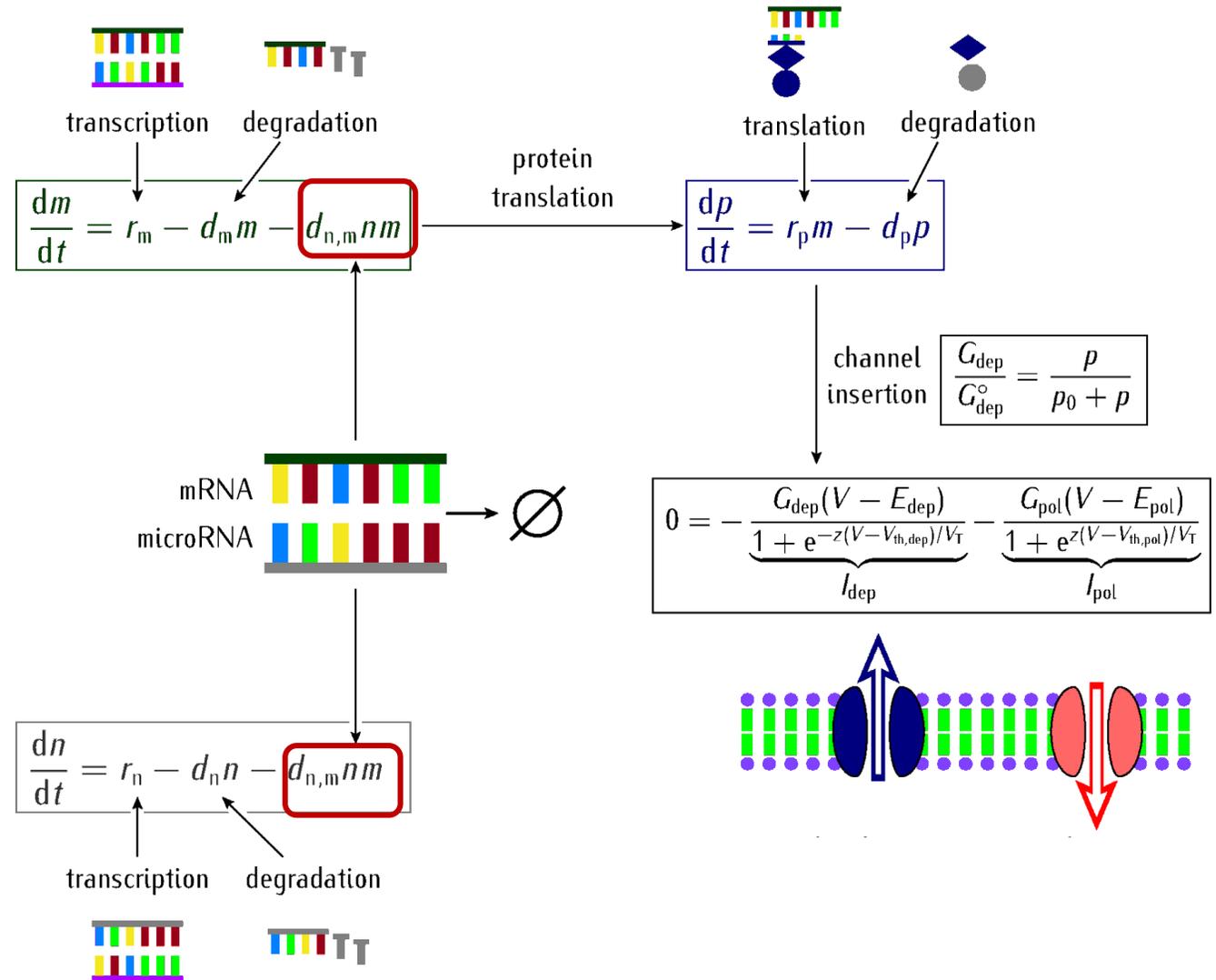
$$C_i \frac{dV_{\text{mem},i}}{dt} = \underbrace{-I_{\text{pol}} - I_{\text{dep}}}_{\text{single cell currents to extracellular environment}} + \underbrace{\sum_{j \in \text{nn}} G_{ij} \cdot (V_{\text{mem},j} - V_{\text{mem},i})}_{\text{gap junction coupling current between neighboring cells}}$$

not only intercellular currents : gap junction-mediated transfer of signaling ions/molecules, e.g. microRNA

miRNA-mediated intercellular communication supported by gap junctions might regulate gene expression in neighboring cells during development.

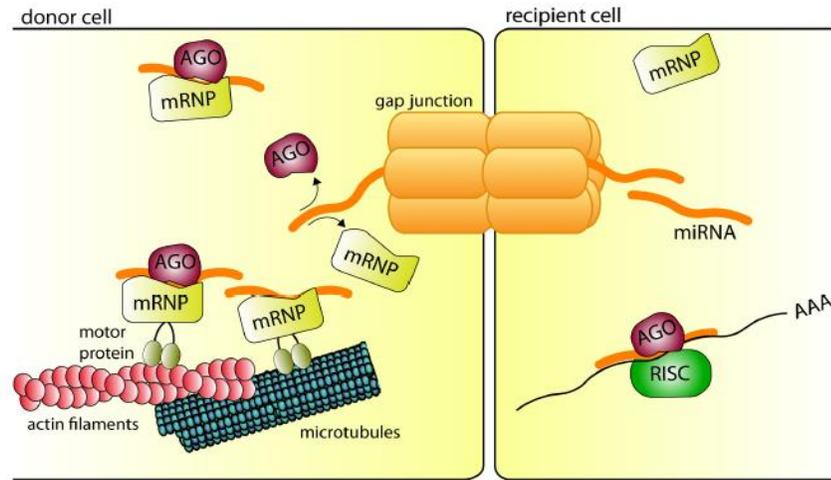
Cell-specific delivery by tuning the junction protein and miRNA characteristics establish spatio-temporal regions with different bioelectrical states.

A biophysical model where the miRNA acts on the mRNA of a specific channel protein thus modulating the expression of the ion channel (right).

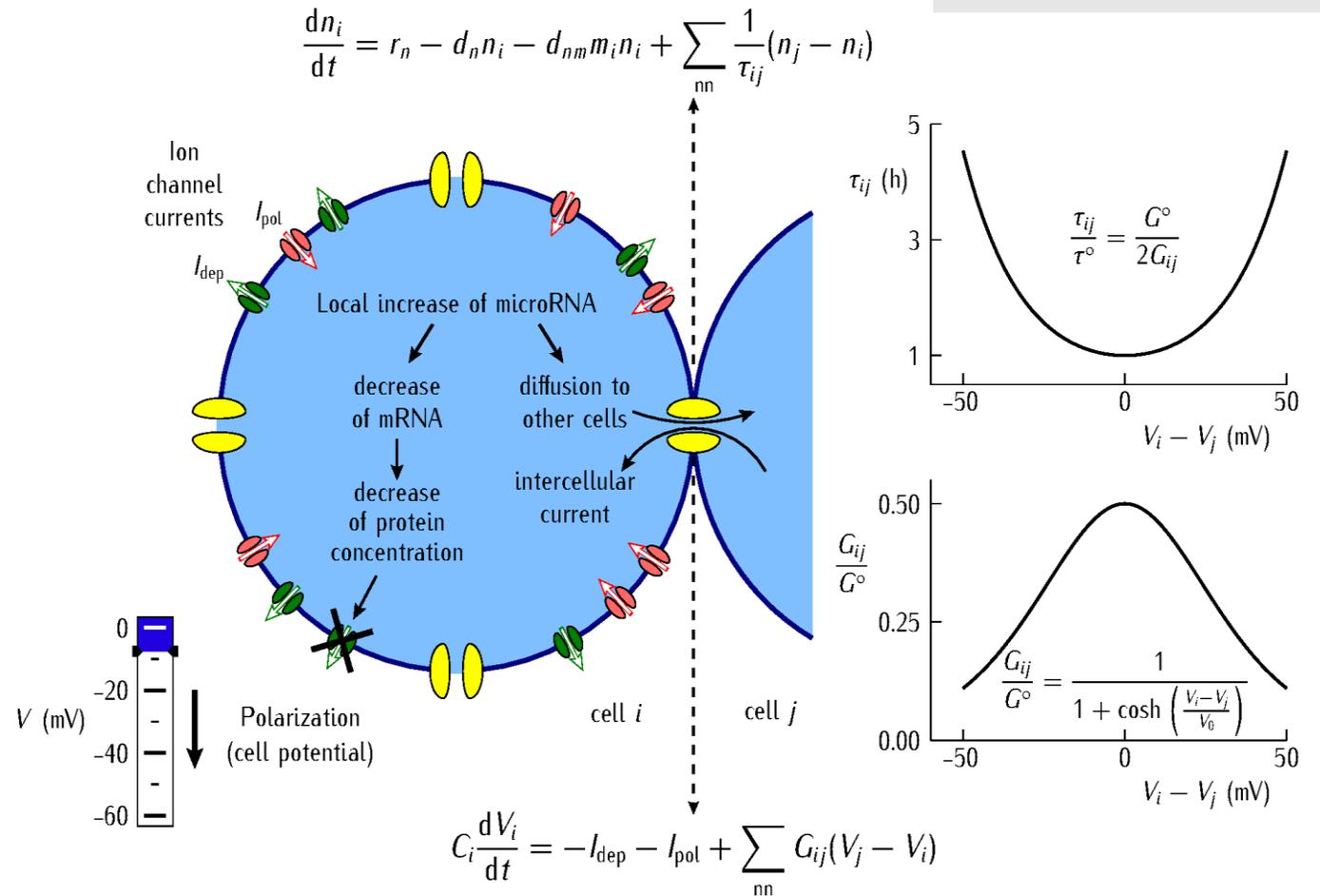


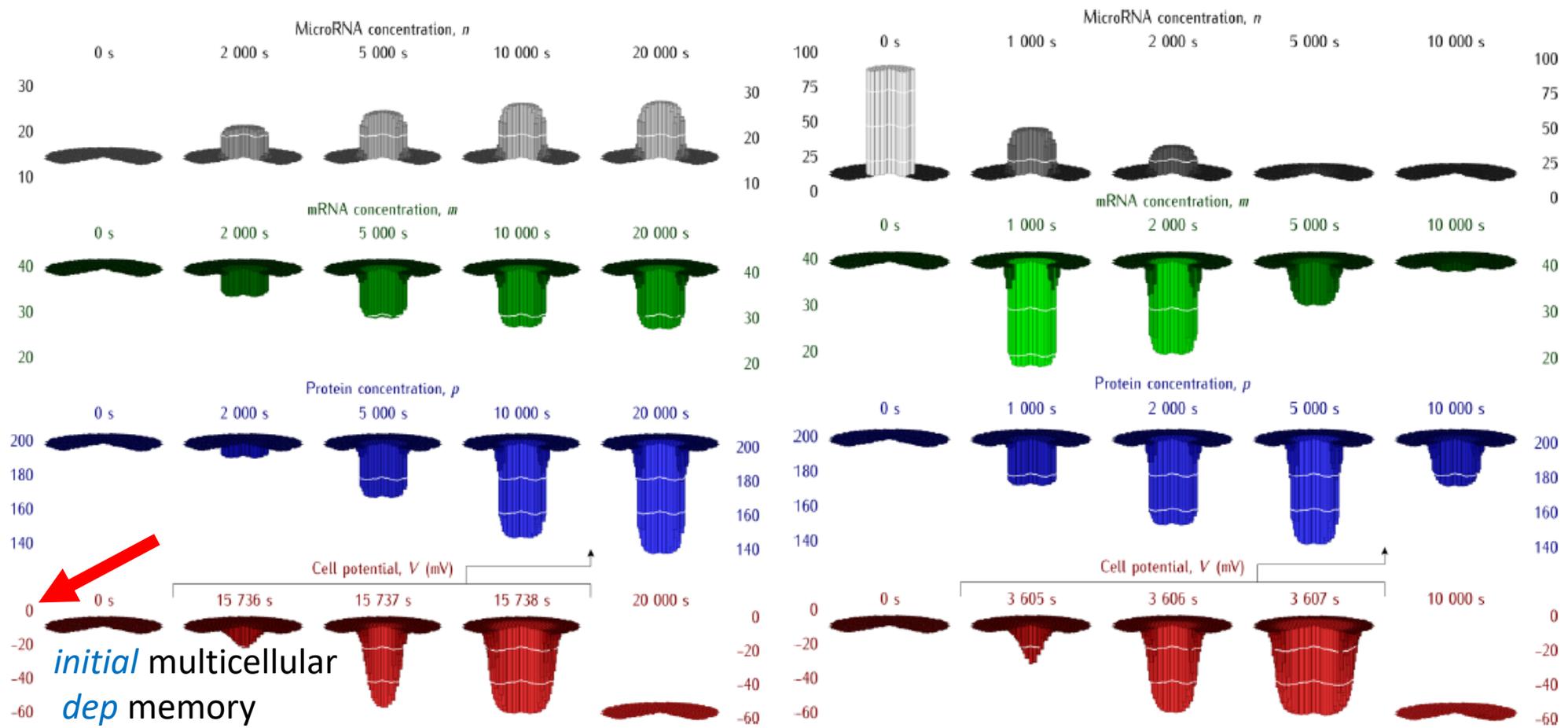
# extended model for microRNA intercellular transference

*J. Phys. Chem. B* 2017  
 10.1021/acs.jpccb.7b04774  
*Traffic*. 2018  
 10.1111/tra.12606



*Translocation of miRNA to gap junctional cell-cell contacts* driven by passive diffusion of miRNA molecules, e.g. bound to AGO proteins or other non-AGO proteins (mRNPs) in the cell.





*miRNA could change a multicellular bioelectrical memory*

*final multicellular pol memory*

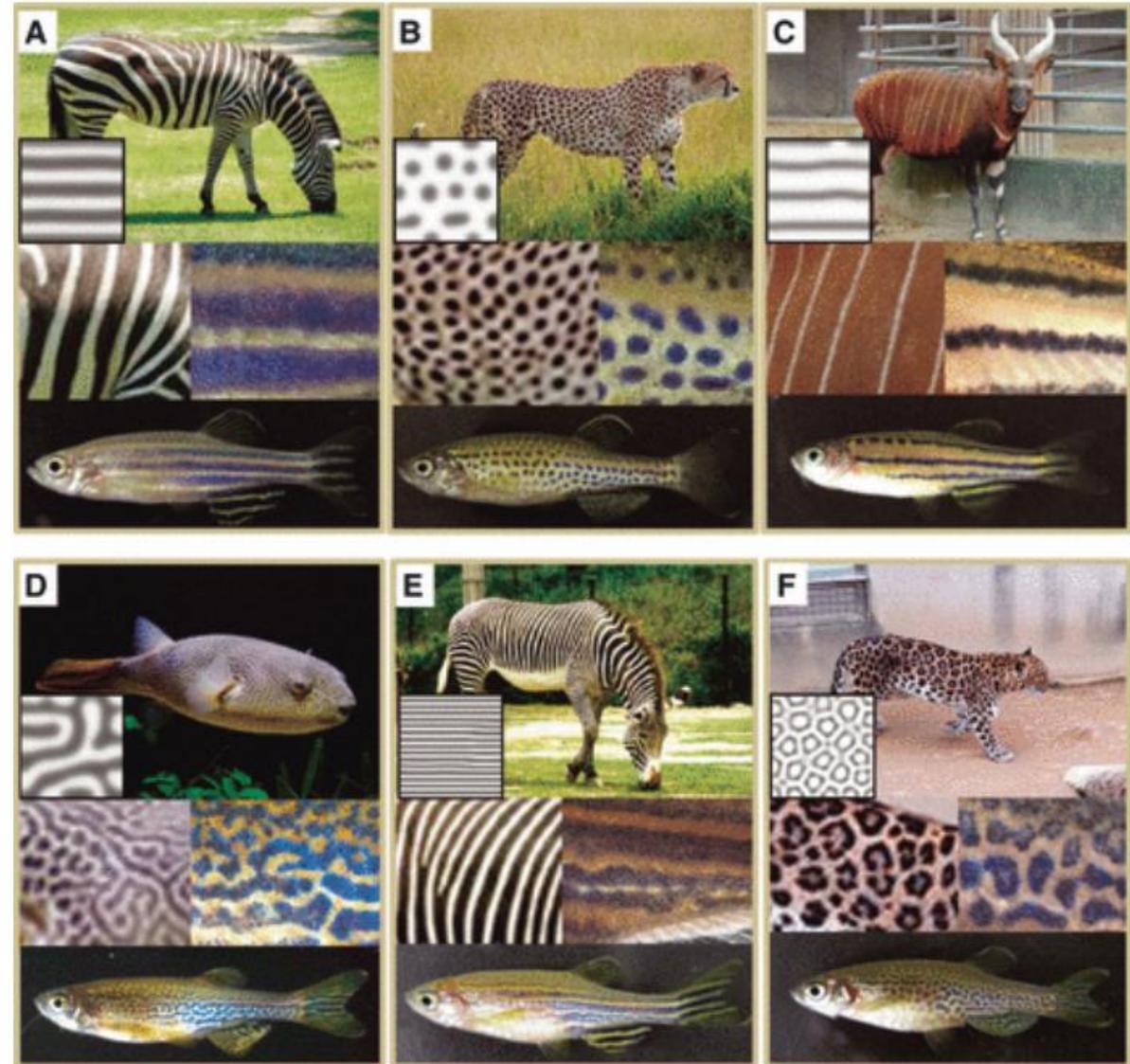
Time changes of *dep* channel *miRNA*, *mRNA*, and *protein* concentrations and the *cell potential* after the local increase of the miRNA transcription rate from  $1.5 \text{ min}^{-1}$  (*external* multicellular domain) to  $2.0 \text{ min}^{-1}$  (*central* domain). The miRNA transference across the intercellular junctions changes the *dep* channel protein concentration, forcing the *repolarization* from the *initially depolarized state* (left). Time changes following a *local transfer of miRNA* over the central domain. Now, the initial *mRNA* and *protein concentrations* are *recovered at long times* because of the finite amount of miRNA transferred but *repolarization is maintained* (right).

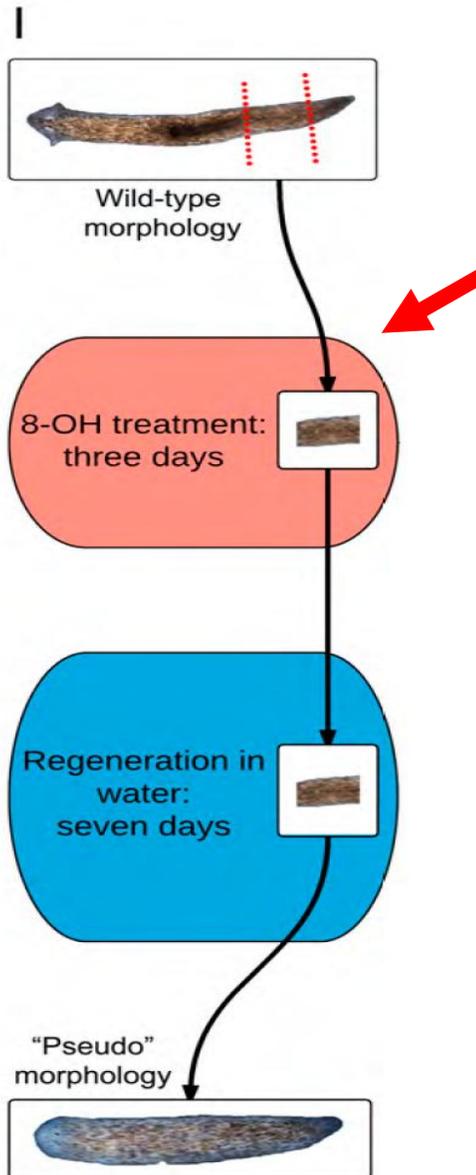
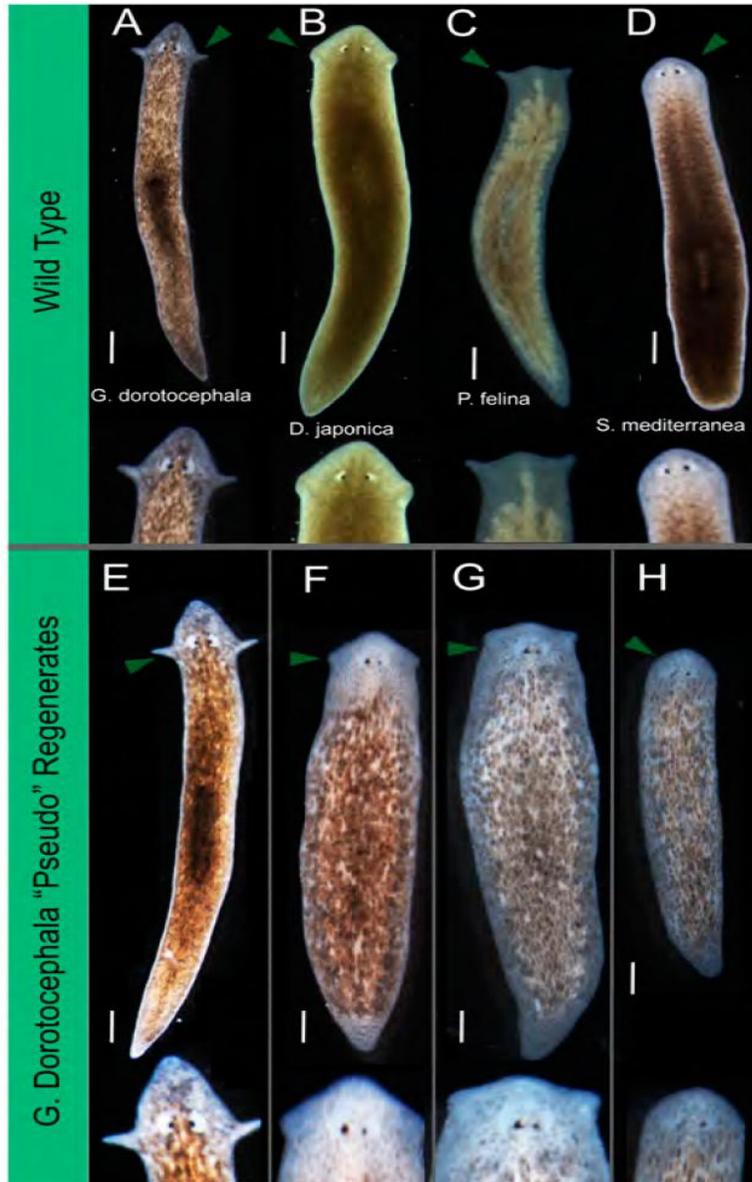
## other experimental systems: gap junction-assisted changing of skin patterns

In a *biochemically-related context*, it has been suggested that gap junctions tune the *reaction-diffusion* (Turing type) processes responsible for the *skin patterns* observed in zebrafish, where slight junction changes lead to pattern diversity.

However, it is difficult to identify the molecules specific to particular cellular processes because of the limited selectivity of the gap-junctions.

*Pigment Cell Melanoma Res.* 2012  
10.1111/j.1755-148X.2012.00984.x  
*J. Biol. Chem.* 2016  
10.1074/jbc.M115.673129

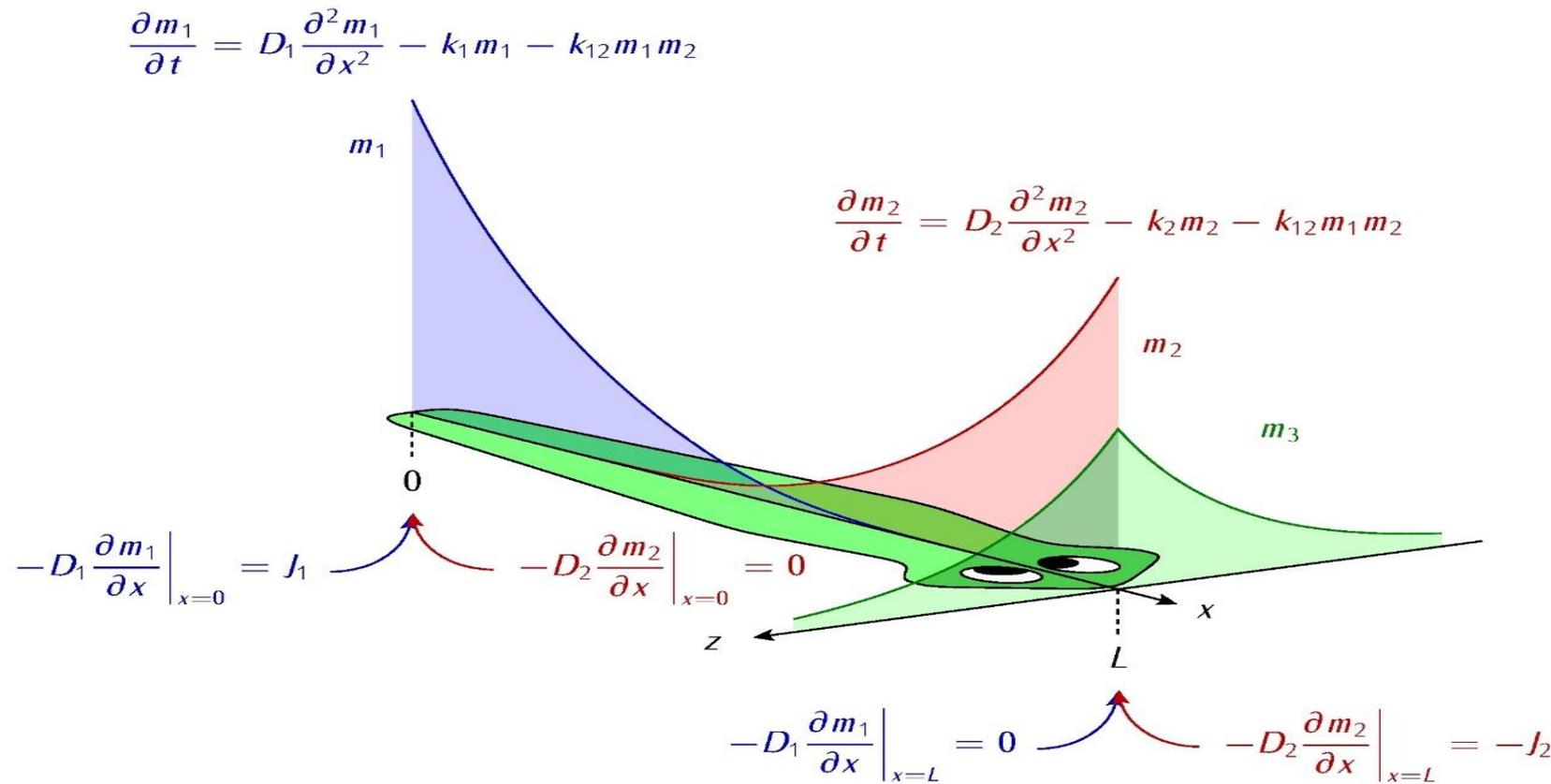




more actions on gap junctions:  
octanol blocking may change  
planaria head morphology

Different *head morphologies* observed  
after *octanol* treatment of *four species*  
of *planaria* flatworm (A–D).

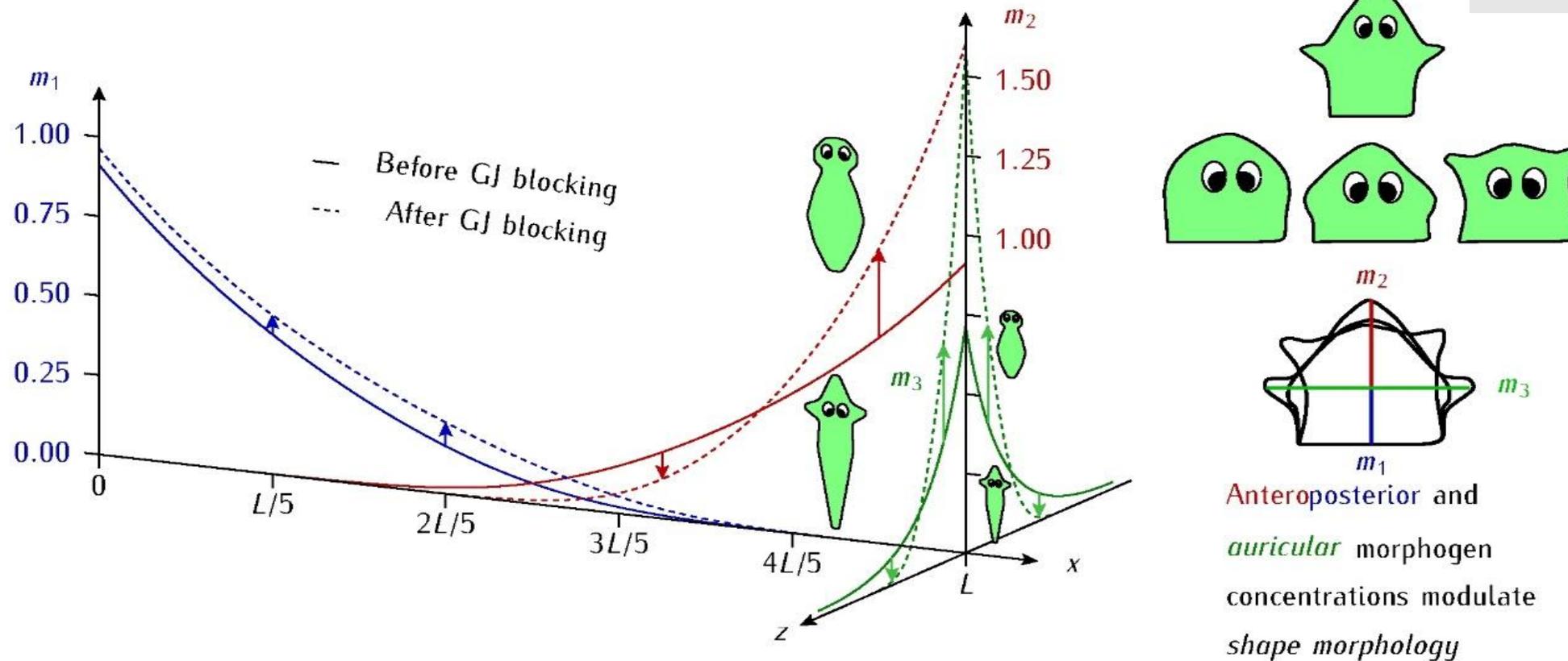
The green arrows indicate the distinct  
head shapes (E–H) obtained when the  
*pre-tail fragments* are *amputated* from  
G. dorotocephala *worms* and treated  
according to the experimental  
procedure. The scale bar corresponds  
to 0.5 mm.



model  
equations  
for planaria  
head  
morphology

Reaction-diffusion equations with boundary conditions at  $x = 0$  (*tail*) and  $L$  (*head*). The *spatial regionalization* of two *morphogen concentrations*  $m_1$  and  $m_2$  along the  $x$  axis establishes the *antero-posterior morphology* of the multicellular system. Because the position of the *central wavefront* between the two concentrations is regulated by the diffusion coefficients  $D_1$  and  $D_2$ , the *gap junction blocking* eventually impacts on the *spatial patterns*. A third morphogen of concentration  $m_3$  that diffuses independently along the orthogonal direction ( $z$  axis) may also influence lateral morphology.

## model qualitative results



Simulations suggest changes in the *instructive patterns* of the antero-posterior morphogens 1 and 2 and the auricular morphogen 3 resulting from the *gap junction blocking*. Do they lead to different *expression patterns* and *head shapes*? This result emphasizes the importance of identifying *biological morphospaces* defined by *experimentally accessible* magnitudes.

## 5. Theoretical results of qualitative relevance

The progress from an *individual cell* to *multicellularity* leads to *average biophysical fields* that influence *development* and *regeneration* (a *high level of abstraction*).

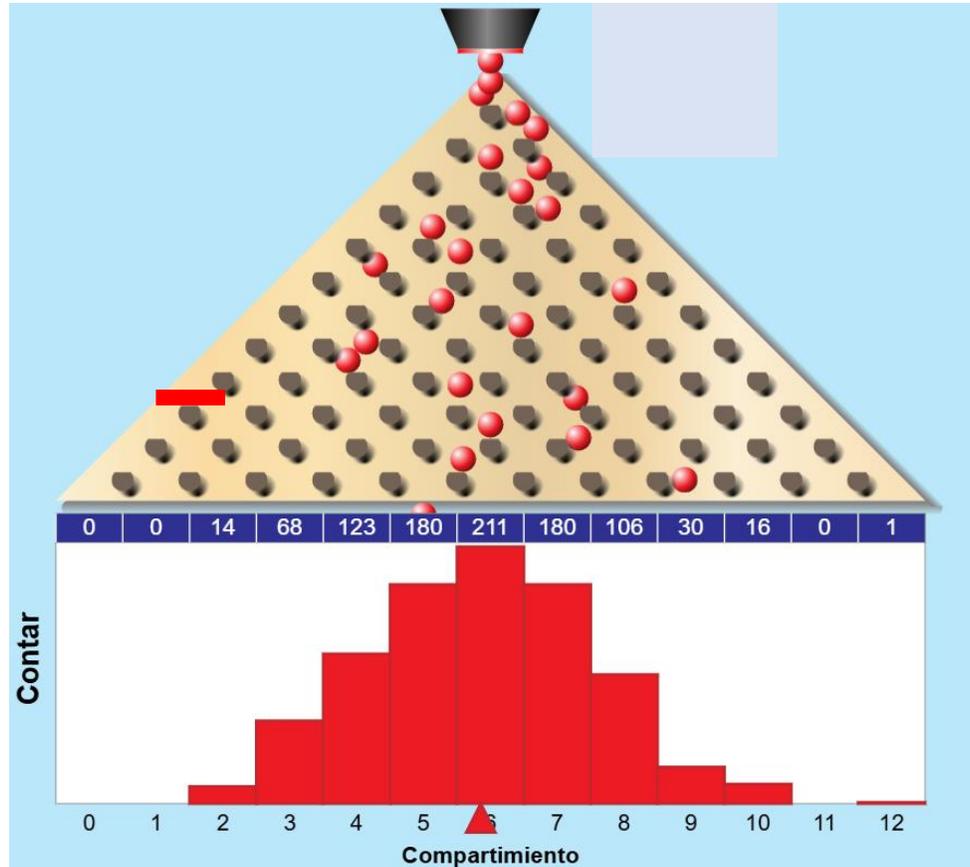
In *Bioelectricity*, an initial

*small asymmetry, local disruptive event, change in environmental conditions*, etc.

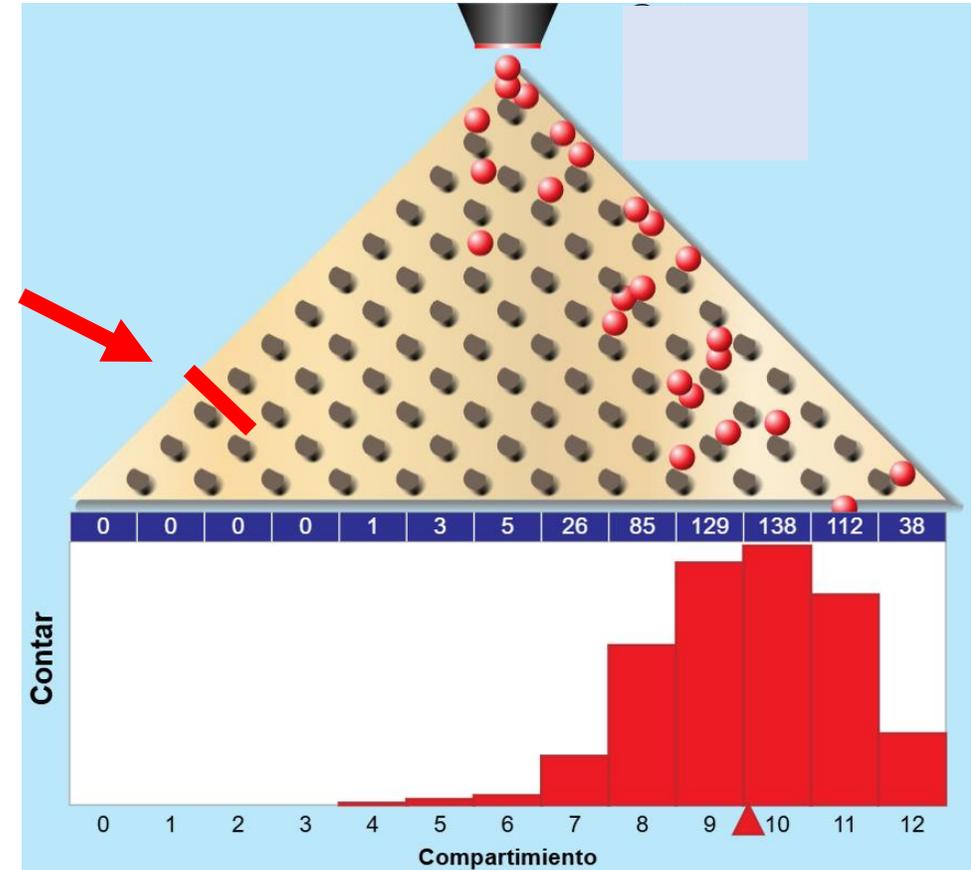
triggers subsequent processes where the coupling between *bioelectricity* and *transcription* may *oppose to* or *amplify* the initial perturbation to *reestablish previous* or *establish new* multicellular programs.

The following *model simulations* explore the *qualitative consequences* of different *symmetry-breaking* and *disruptive* phenomena, with an emphasis on *bioelectricity*. Other *biochemical* and *biomechanical* processes not included here may also be relevant in real systems.

A previous observation: *complex phenomena* such as *symmetry-breaking* and *shape regulation* can arise in relatively *simple systems* composed by multiple units.

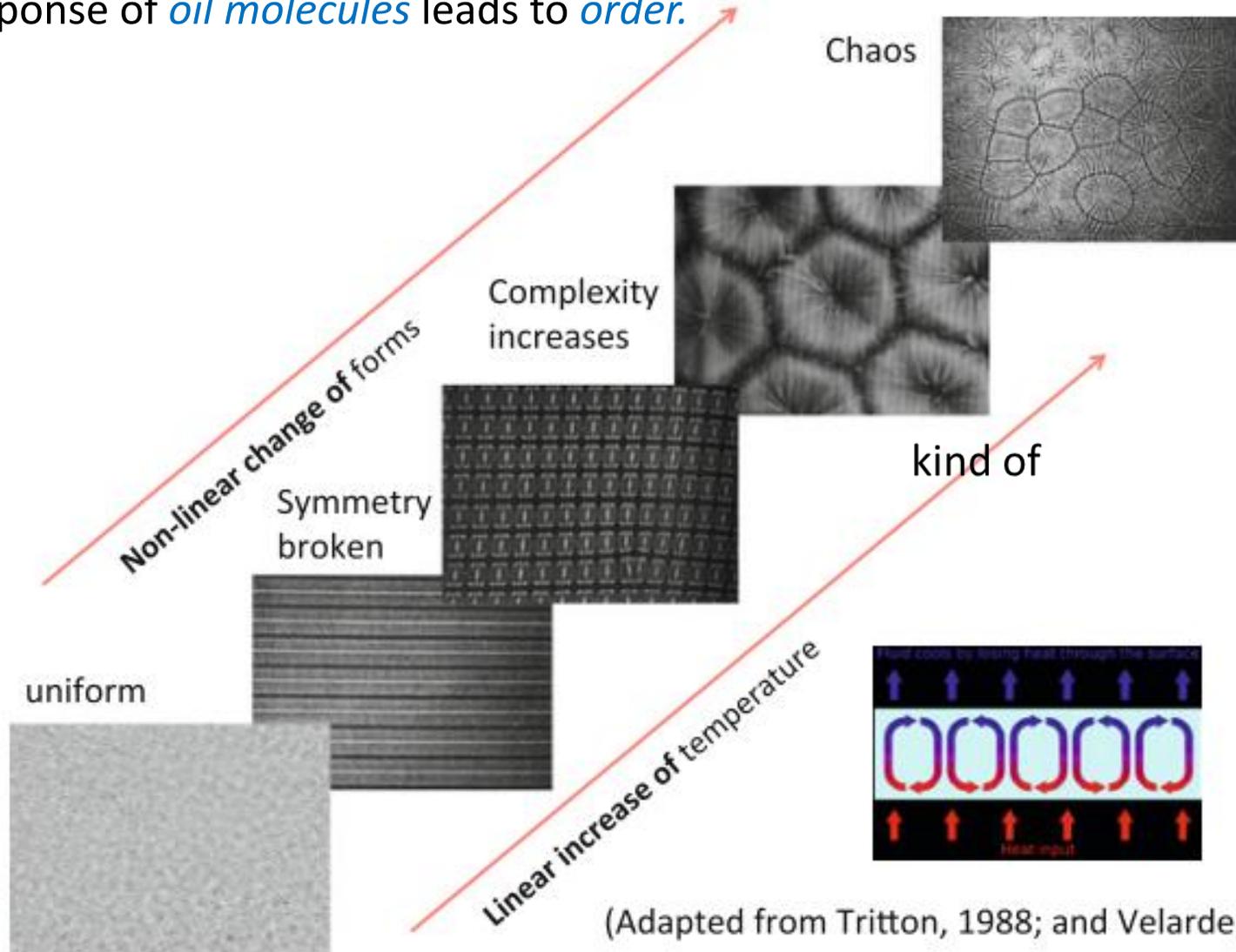


*central*



*left-right*

Rayleigh-Bénard cells: the *collective* response of *oil molecules* leads to *order*.



When heating from below a thin layer of oil spread out on a plane surface, *convection currents* with peculiar geometric shapes (lines or polygons) *self-organize*.

The shapes change abruptly when the temperature gradient goes over particular *thresholds*.

This non-linearity may *mimic* self-organization in *living systems*

[Photos adapted from Tritton (1988); and Manuel Velarde, Universidad Complutense, Madrid].

In contrast to the *universality* of equilibrium phase transitions, *no general theory* predicts what organization arises far from equilibrium in *biological systems*: systems have *specific details* that matter.

However, two *bioelectrical features* are central to our model:

*Contrast dynamic sharpening*: *channels* and *gap junctions* operate over *wide voltage windows*; they may *polarize* or *depolarize* depending on the cell state and the neighboring conditions, providing the spatio-temporal contrast needed for *bioelectrical regionalization*.

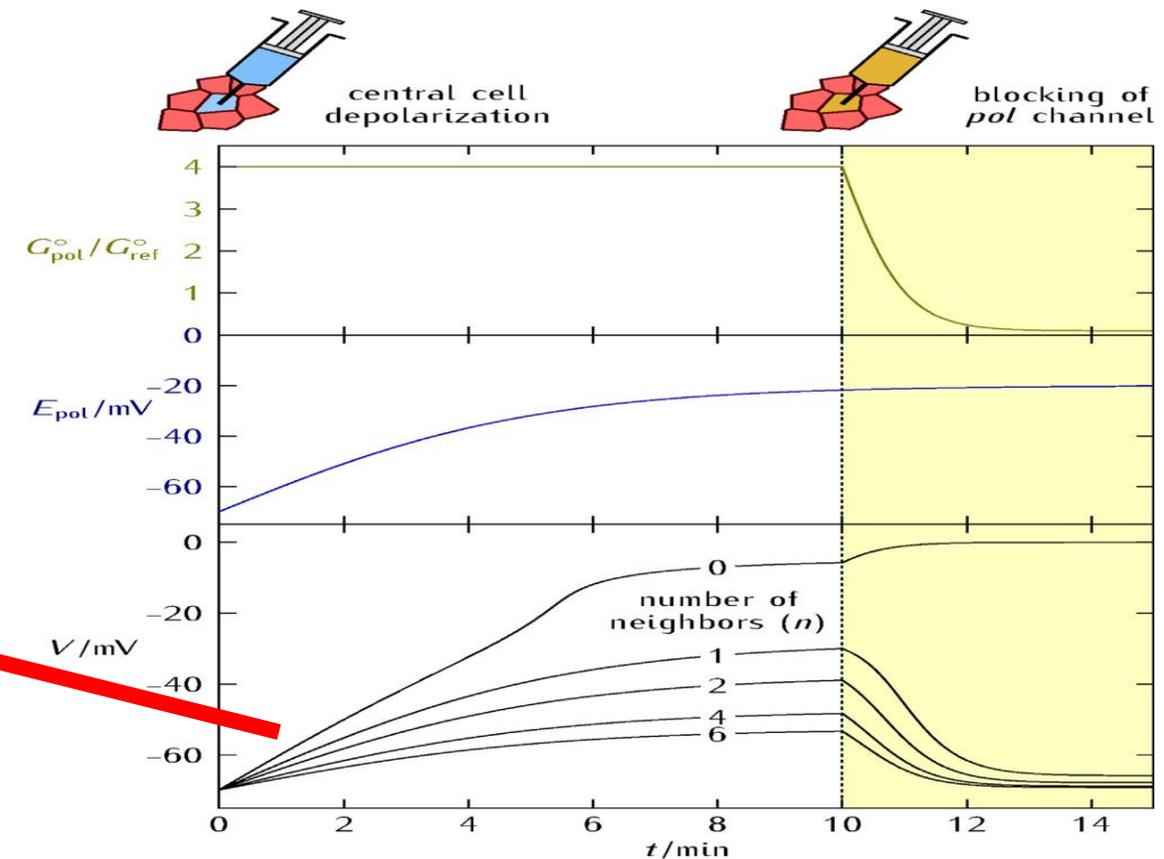
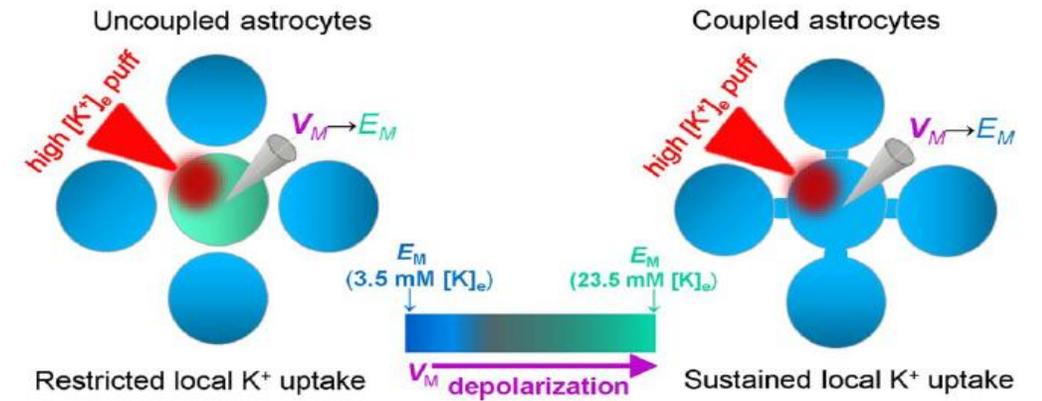
*Degraded pattern recovering*: “corrupted images” due to a *local cell depolarization/polarization* (single-cell failure) or a *decrease of connectivity* (intercellular failure) can still be recovered by *single-cell* (e.g. channel redundancy re-establishes the normal cell state) or *multicellular* (e.g. community effects re-establish intercellular communication) mechanisms.

## the bioelectrical community effect

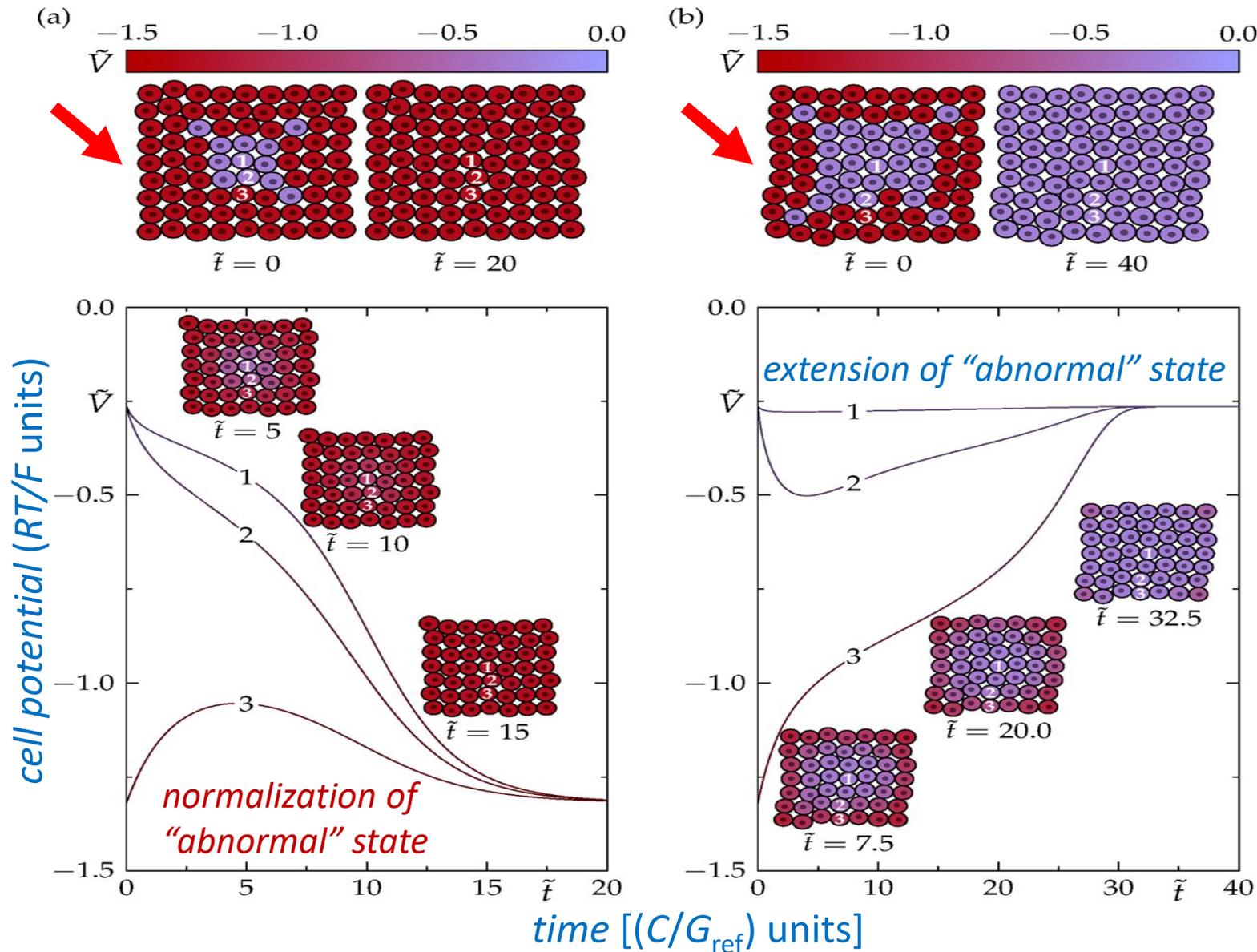
The *gap junction-mediated* transfer of *electrical* and *chemical* signals in a *multicellular community* provides a *feeling of belonging* to a cluster.

The *buffer effect* due to the *neighboring cells* can *correct* a perturbation, which is caused here by the *abnormal depolarization* of the *central cell* due to a local change in  $E_{\text{pot}}$  (it could also be caused by the *pol* channel *blocking*). The community effect *opposing* the *disruptive* event increases with the *number of neighbors* and the *junction conductance*.

**Important:** each central cell *bioelectrically* “*measures*” the *number of neighbors* ←  
–not only biomechanics!



## a bioelectrical community effect



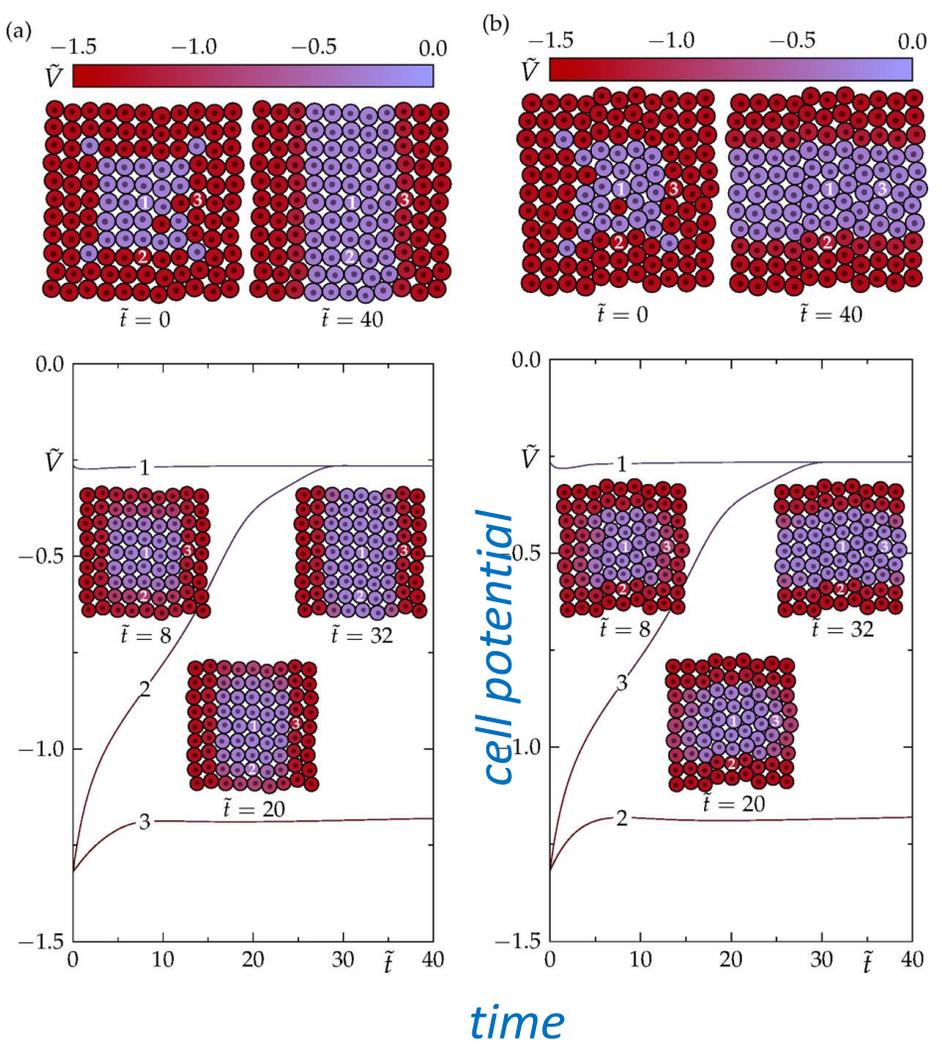
Bioelectrically *suppressing* (left) or *enhancing* (right) a disruptive local *depolarization* event:

for *small regions* of “*abnormal*” cells, the community effect allows a *normalization* of the bioelectrical cell state (left).

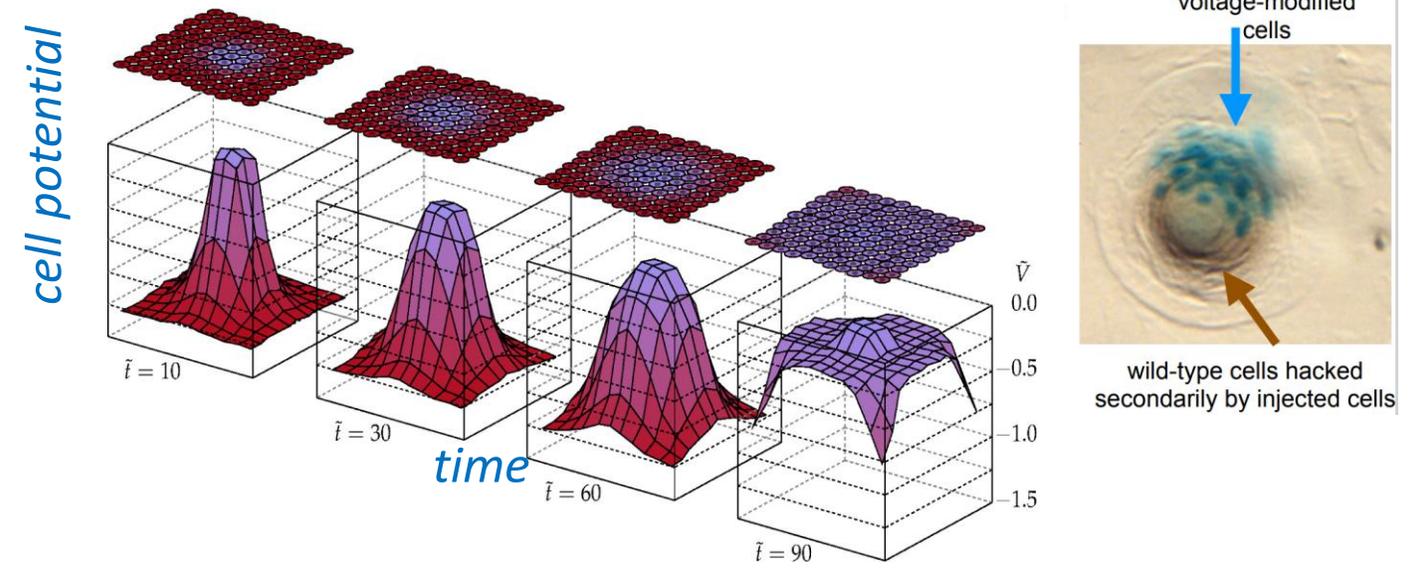
This normalization is *not possible* for *large “abnormal” regions* (right).

Thus, the *intercellular connectivity* could *normalize small regions* but also *spread the abnormal cell state* in the case of large enough regions.

A *pre-pattern* of intercellular *gap junctions* allows imprinting a *directionality* to the tissue *re-writing*.

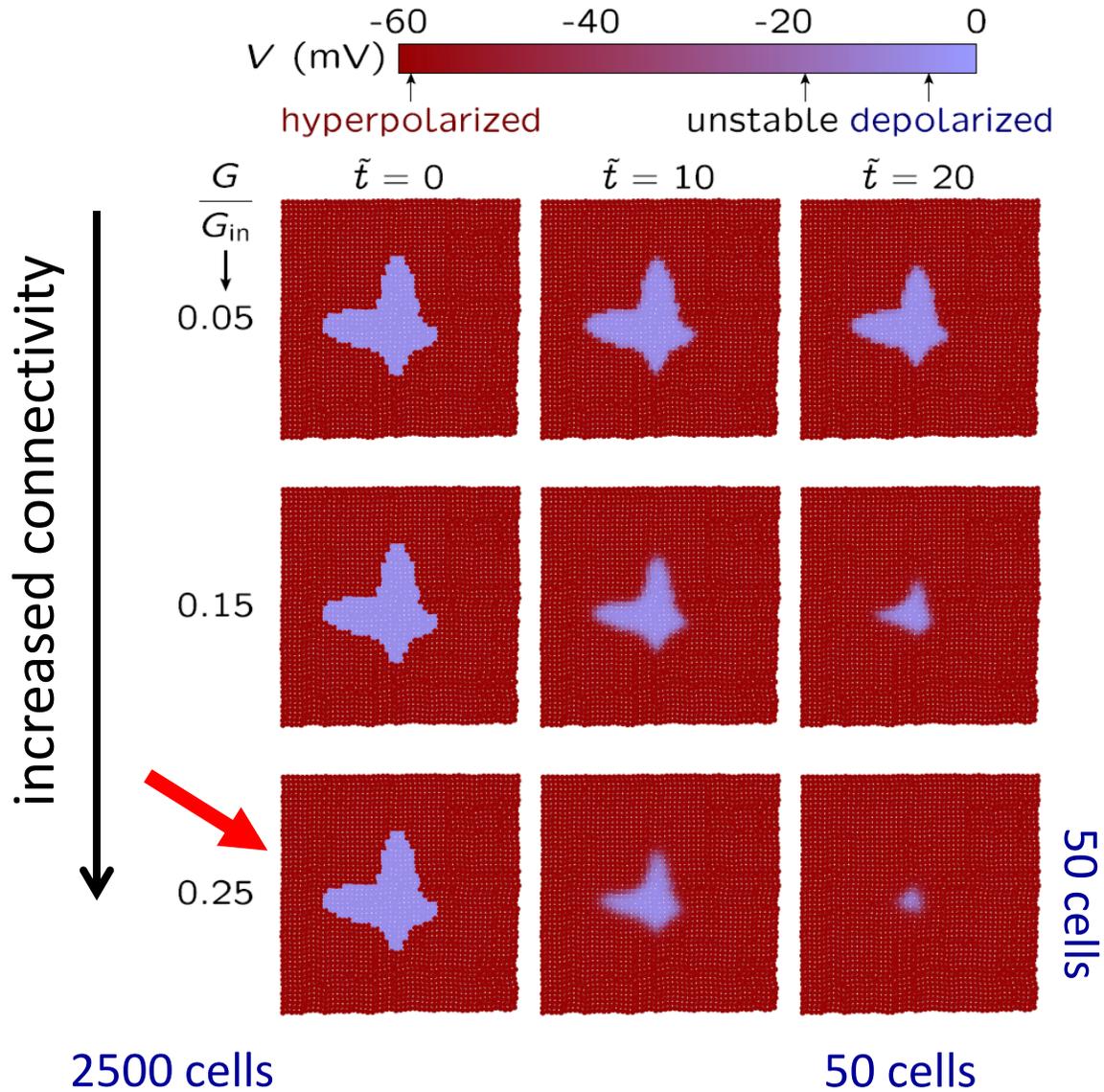


*bioelectrical recruiting* by *depolarizing wave*?



A *patch of cells* in a depolarized (*blue*) state, established by a pulse current, a mis-expression, or a blocking of the *pol* potassium channel, recruits the initially polarized (*red*) neighbors to *re-write* a *bioelectric pattern*.

Operational limitation: this recruiting is *not* always possible because it depends critically on the *relative stability* of the model *pol* and *dep* cell states.



### stage-dependent behavior of intercellular junctions:

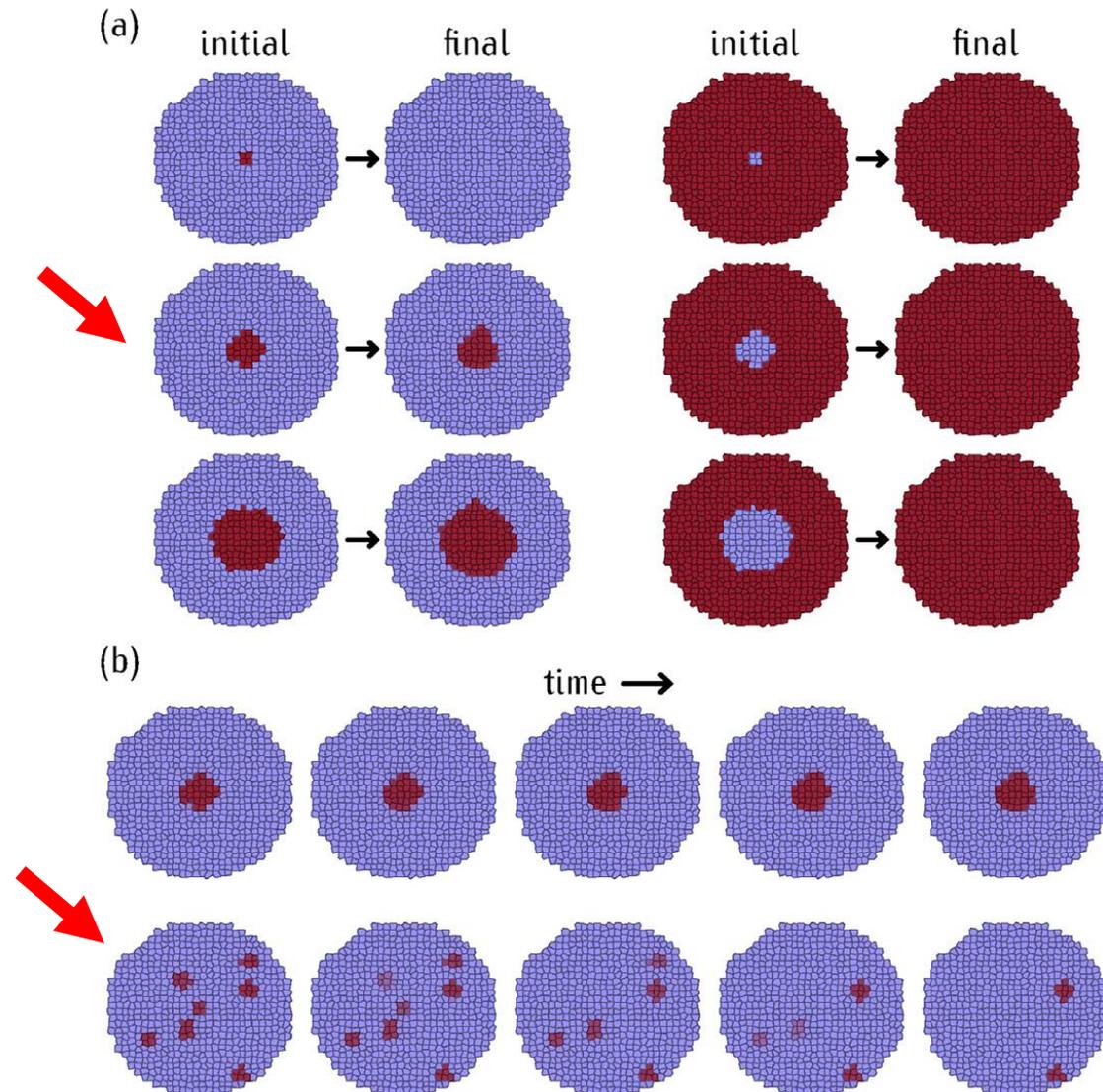
- at *early* stages, a disruptive event could be *bioelectrically suppressed* by *increasing* the connectivity of the *small abnormal* region with the normal neighborhood.

However,

- at *final* rather than initial stages, the disruptive event should be *suppressed* by *decreasing* rather than increasing the *internal* connectivity (*cohesion*) of the now *large abnormal* region, as shown in [10.1103/PhysRevE.102.052412](https://doi.org/10.1103/PhysRevE.102.052412)).

**Experimental limitations:** 1) not only *electrical* but also *chemical* signals are transferred through gap junctions; and 2) bioelectrical *suppression* or *enhancement* of a disruptive event depends on the *relative* stability of the *pol* and *dep* cell states.

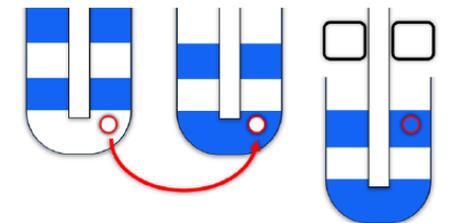
Phys. Rev. E 2020  
 10.1103/PhysRevE.102.052412  
 Commun. Biol. 2024  
 10.1038/s42003-024-06037-4



(a) The *community* effect is *size-sensitive* and depends also on which *isolated cell state* is dominant at the *single-cell* level: an “abnormal” *initially depolarized central region* can be *normalized* (right) but a polarized central region could resist the community normalization effect (left) because the *pol* state is dominant over *dep* state in this simulation.

(b) A *big patch* of cells shows a *stronger community* effect than many *small patches*: small bioelectrically “abnormal” regions hardly progress within a *highly-connected* multicellular ensemble.

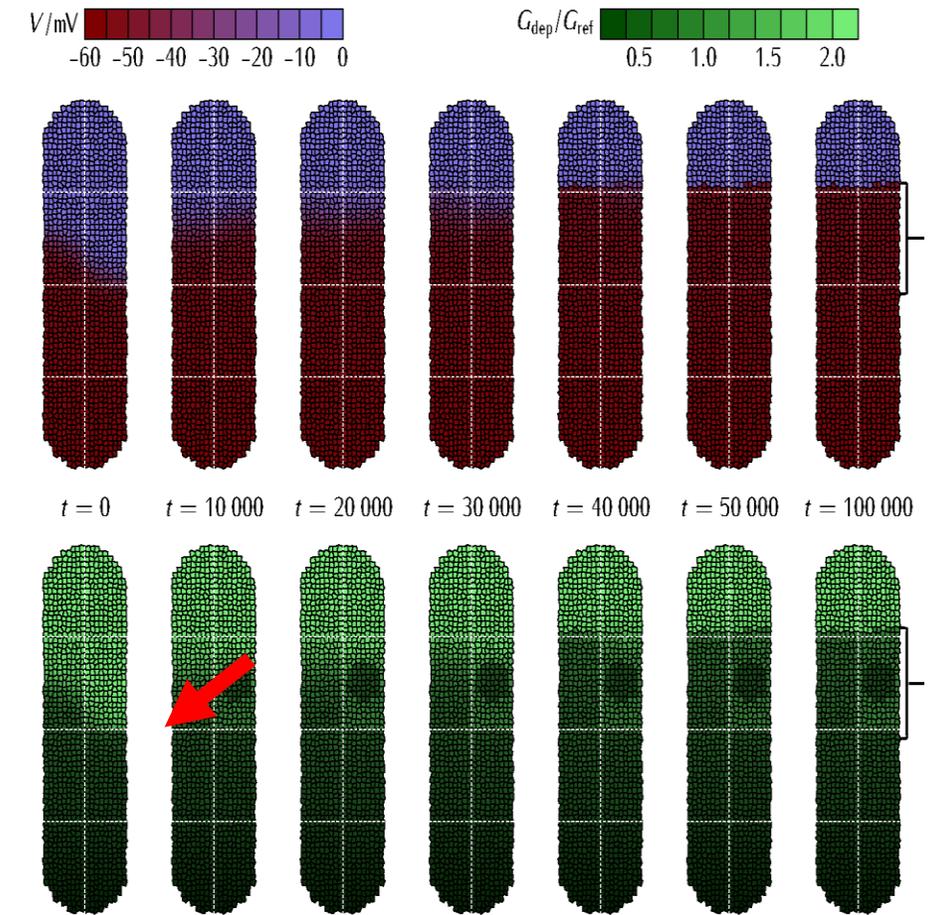
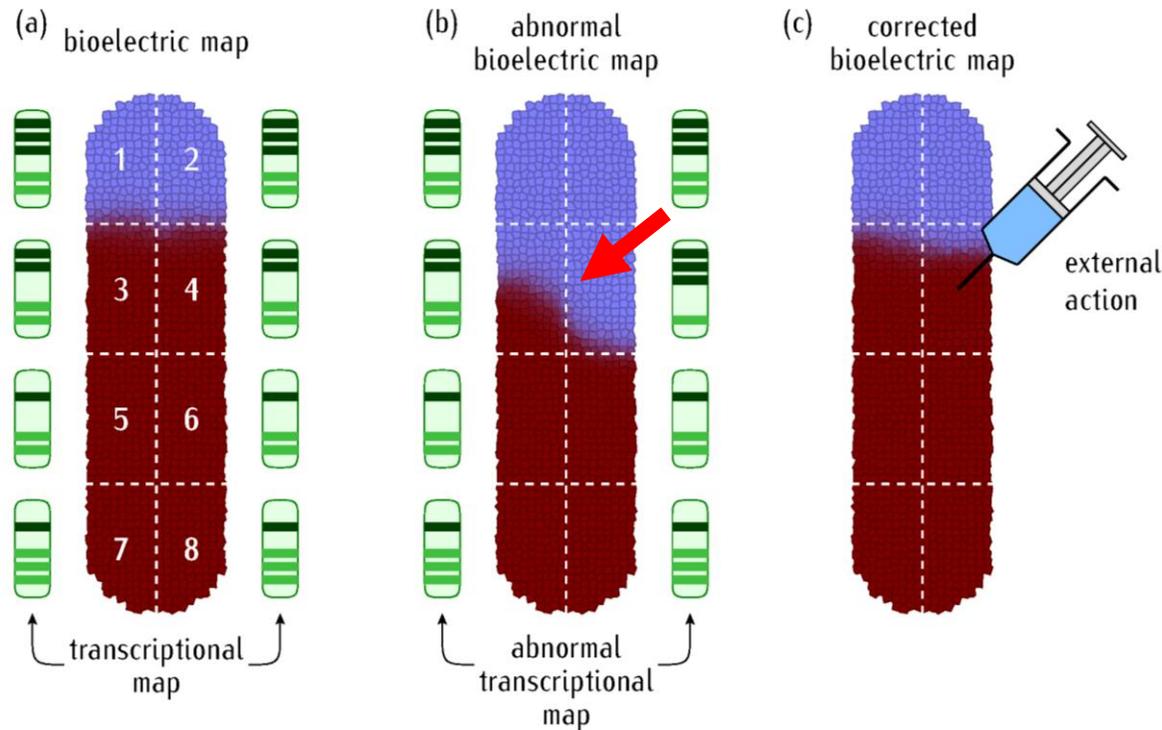
A *biochemical* reminiscence (cell grafting in *segmentation*)?



## correction and completion: can locally degraded patterns be restored?

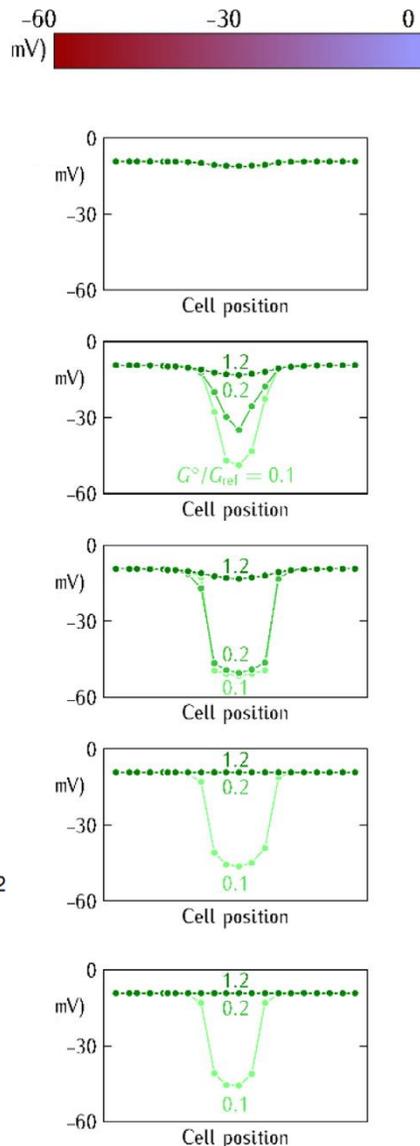
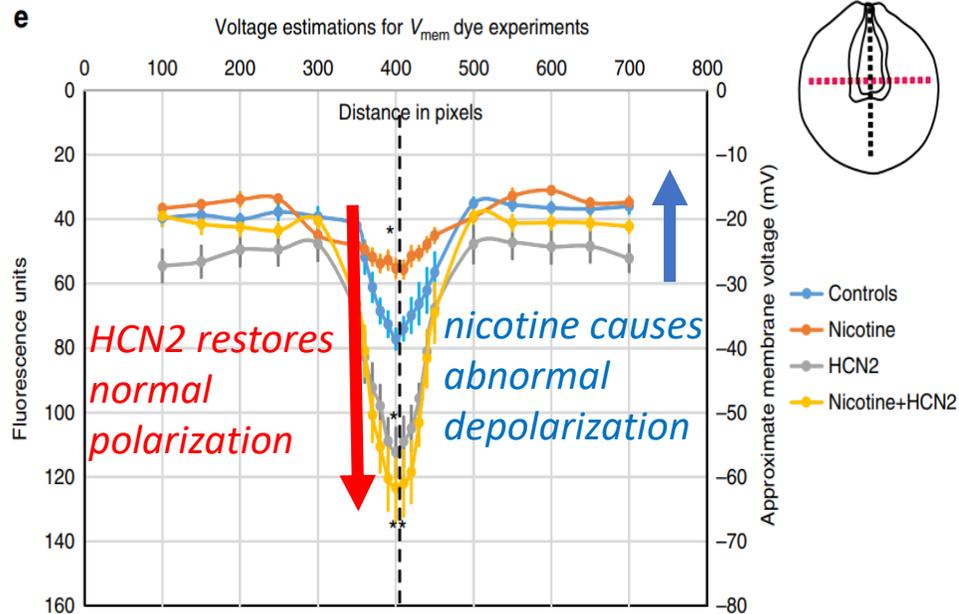
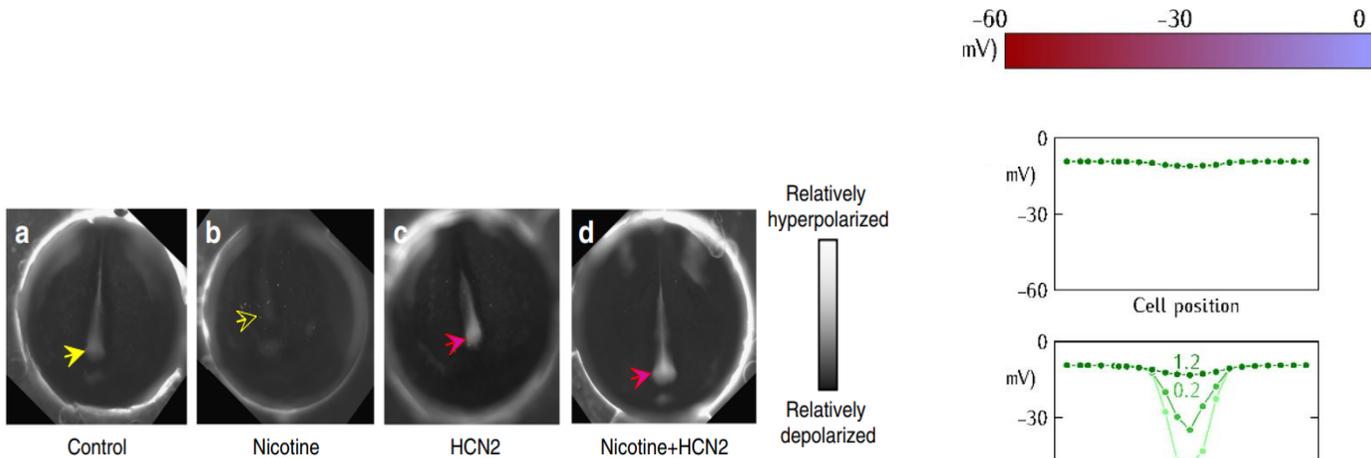
Because of the *coupling* between *bioelectricity* and *transcription*, the simulations suggest that *corrupted spatio-temporal maps* could be *corrected* by *external actions*:

the *blocking* of an *incorrect (dep)* channel or the *upregulation* of a *rescue (pol)* channel *restores* the *correct pattern*.

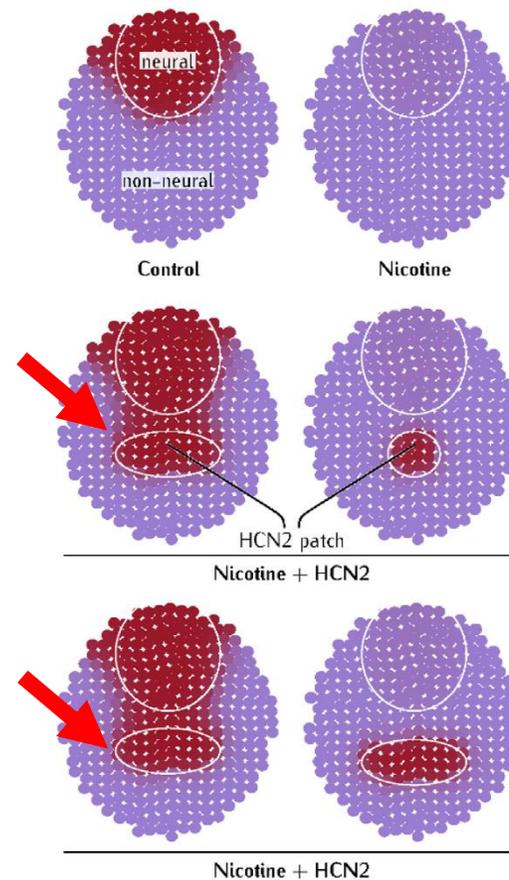


relative potentials between separated cell groups influence transcriptional patterns: are distant actions (polarization waves) possible?

Nat. Commun. 2018  
10.1038/s41467-018-03334-5  
Front. Cell. Neurosci. 2020  
10.3389/fncel.2020.00136



(b) distant cell bioelectrical recruiting

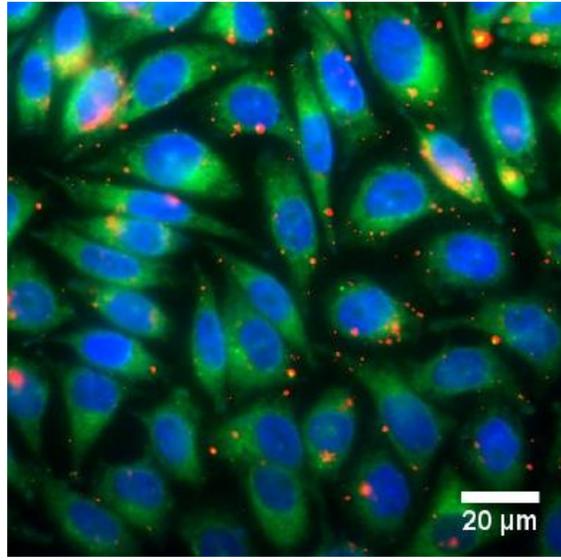


Incorporation of a polarizing channel (HCN2) partially recovers, *at distance*, the *potential pre-pattern* partly distorted by previous nicotine exposition.

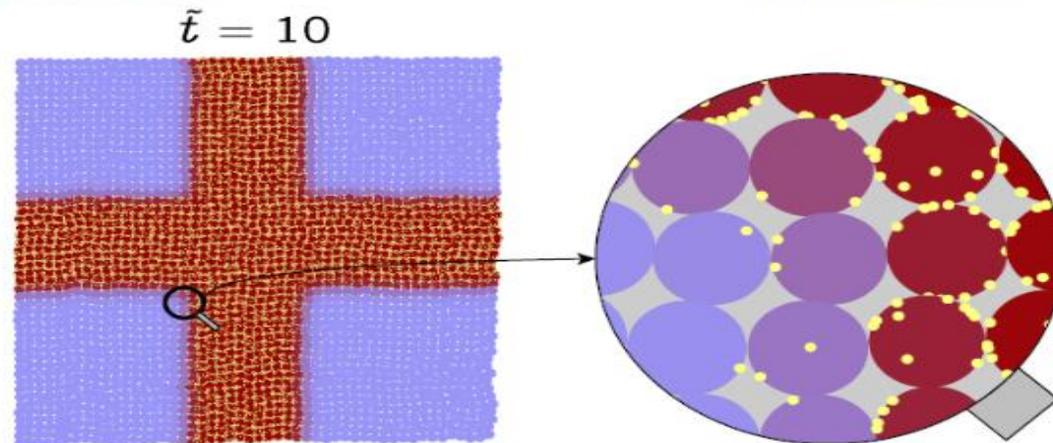
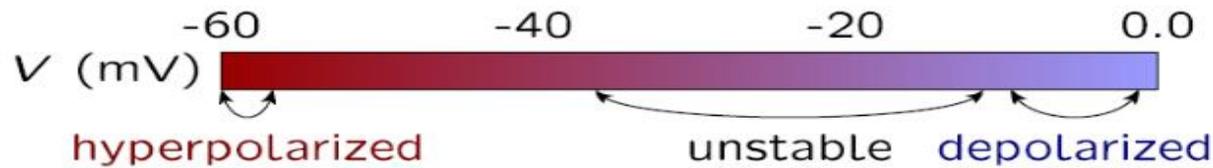
Size and *distance* effects influence the *repolarization*.

## are other correcting actions possible (nanoparticles seeding)?

Nanoscale 2013  
10.1039/C3NR01667F  
RSC Adv. 2015  
10.1039/C4RA15727C  
Small 2024  
10.1002/smll.202404152  
Sci. Rep. 2016  
10.1038/srep20403  
Phys. Rep. 2023  
10.1016/j.physrep.2022.12.003



The 60 nm *amine-modified polystyrene nanoparticles* (small red circles) bound to the *Chinese Hamster Ovary (CHO) cell membranes* can be observed by flux cytometry, dyes, and fluorescence microscopy techniques (*up*).

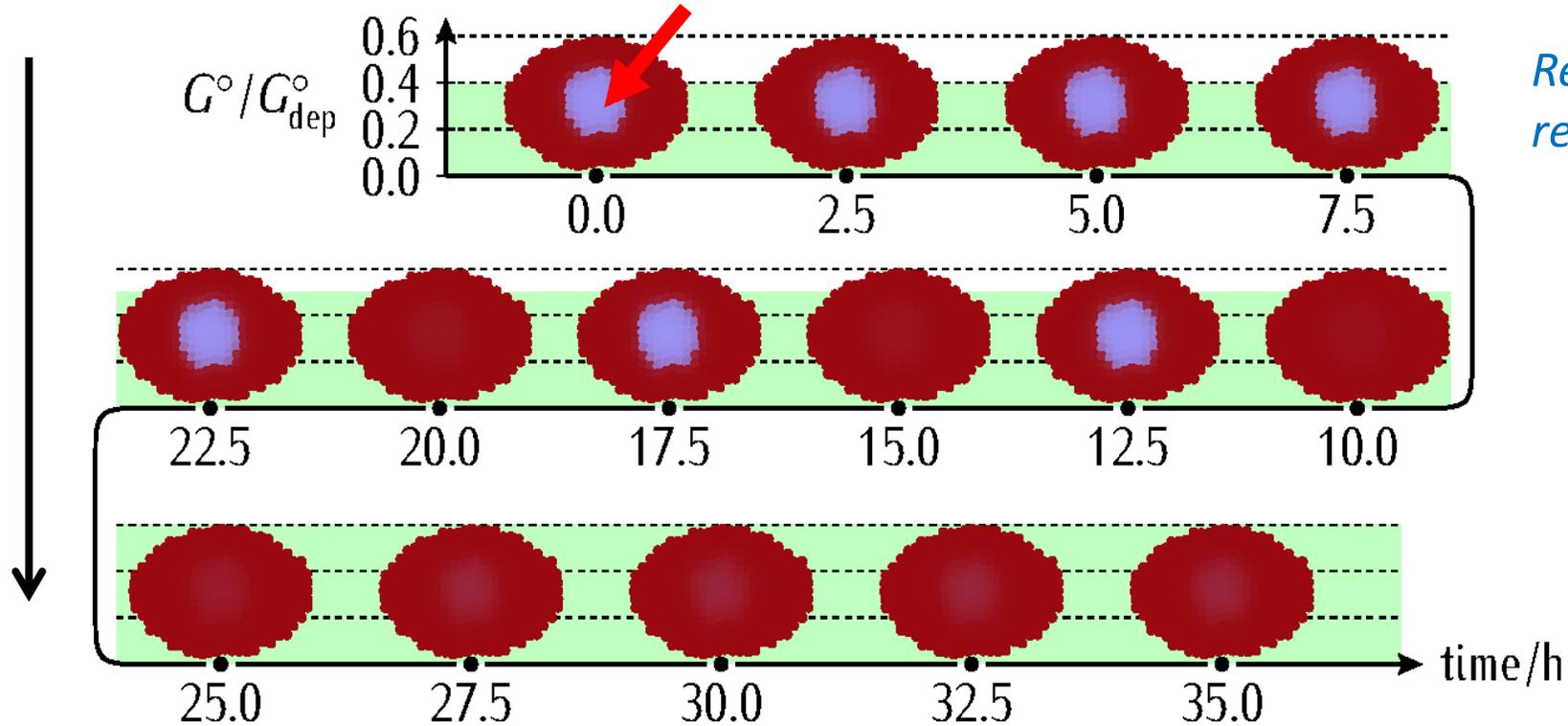


The bioelectrical simulation suggests that the *spatial distribution* of *positively charged nanoparticles* (small yellow circles) follows the *cell potential pattern*: particle accumulation is more significant around polarized (*more negative*) cells than around depolarized cells. The inset zooms a small region of the multicellular system (*down*).

Limitation: not only electrical but also *hydrophobic/hydrophilic* contributions to *nanoparticle binding*.

## oscillatory multicellular potentials: acting on the intercellular connectivity

Increasing connectivity



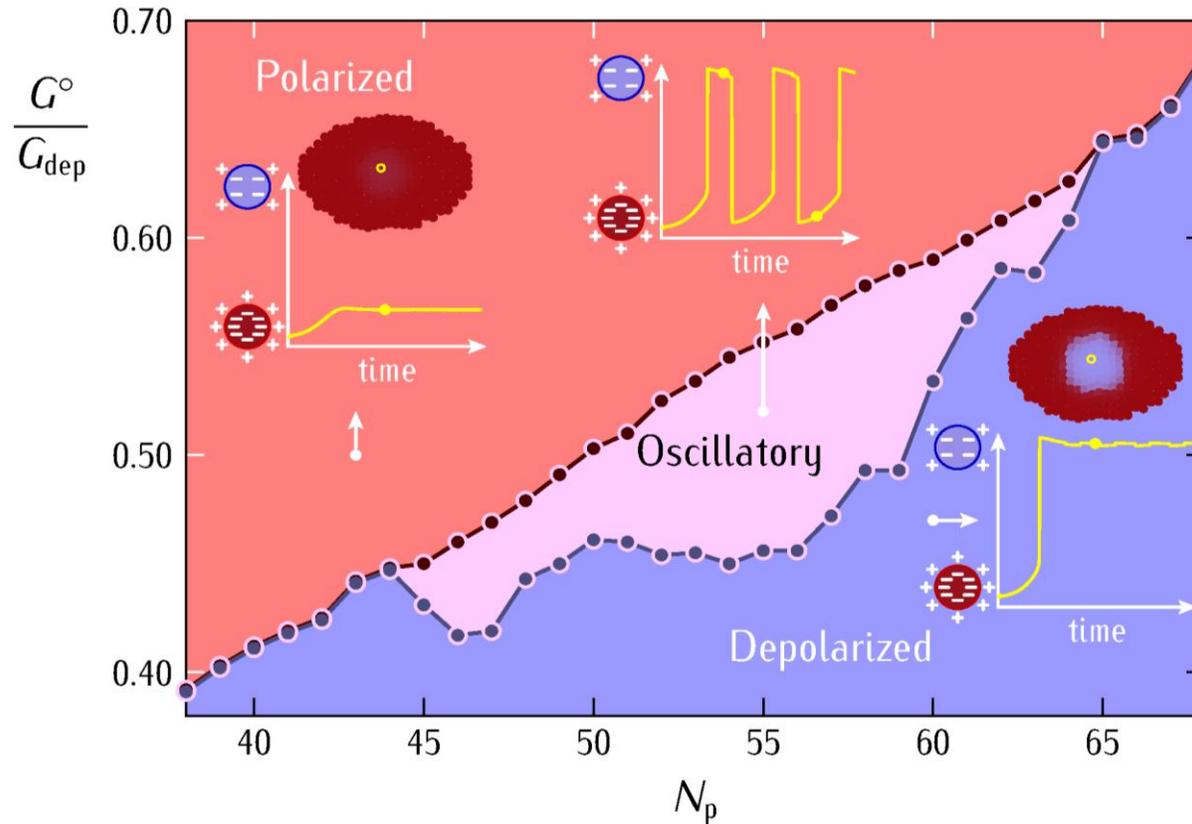
Regionalization by exogenous grafted region or endogenous disruptive event

Collective oscillation

Normalization: isopotential system

Transitions between *depolarized* and *polarized* multicellular states, including *collective oscillations*, can be modulated by the *intercellular connectivity*. Note that *three* possible bioelectrical states can be realized in the central region, which suggests that external *instructive actions* on the *gap junctions* should be possible.

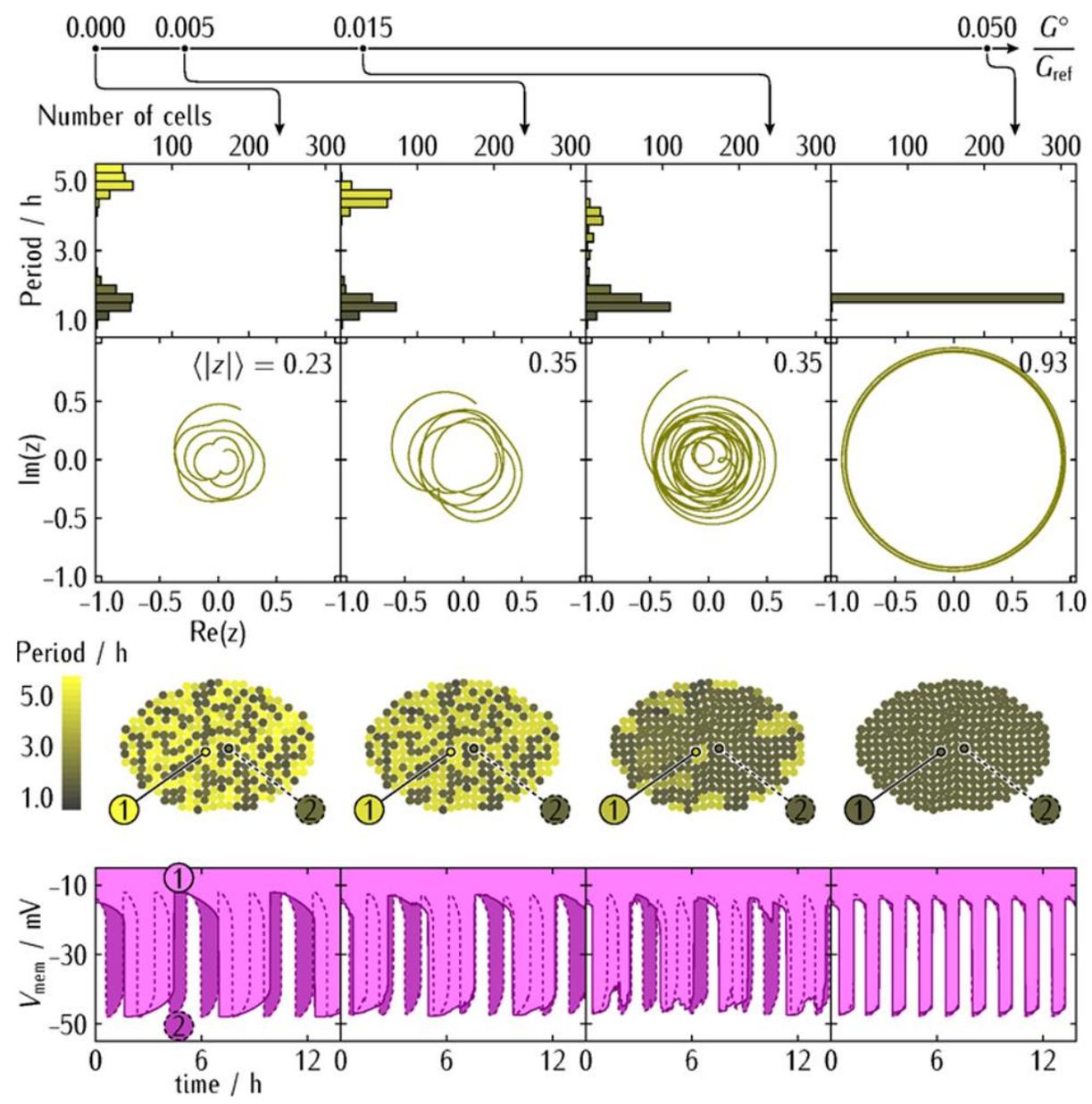
*configuration phase space for central region: different bioelectrical states can be established by changing intercellular connectivity*



*important:* change in the external or endogenous conditions  $\rightarrow$  change in the bioelectric-genetic coupling  $\rightarrow$  change in the multicellular configuration space

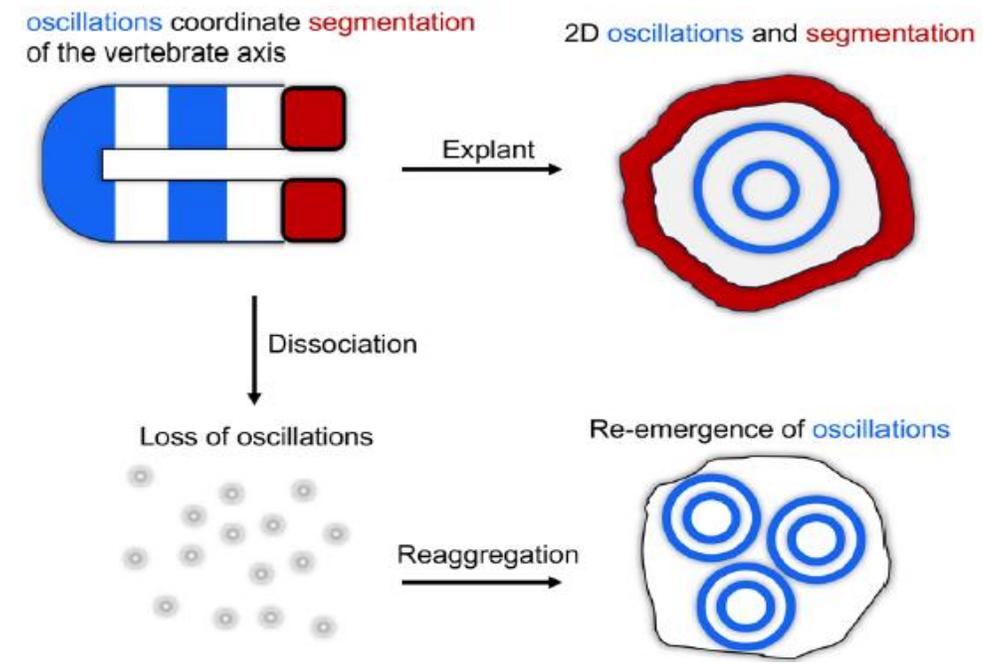
Polarized, depolarized and oscillatory *multicellular states* as a function of the intercellular *junction conductance* and the *number of cells* in the central region: exploring the *bioelectrical* rather than *genetic* phase space.

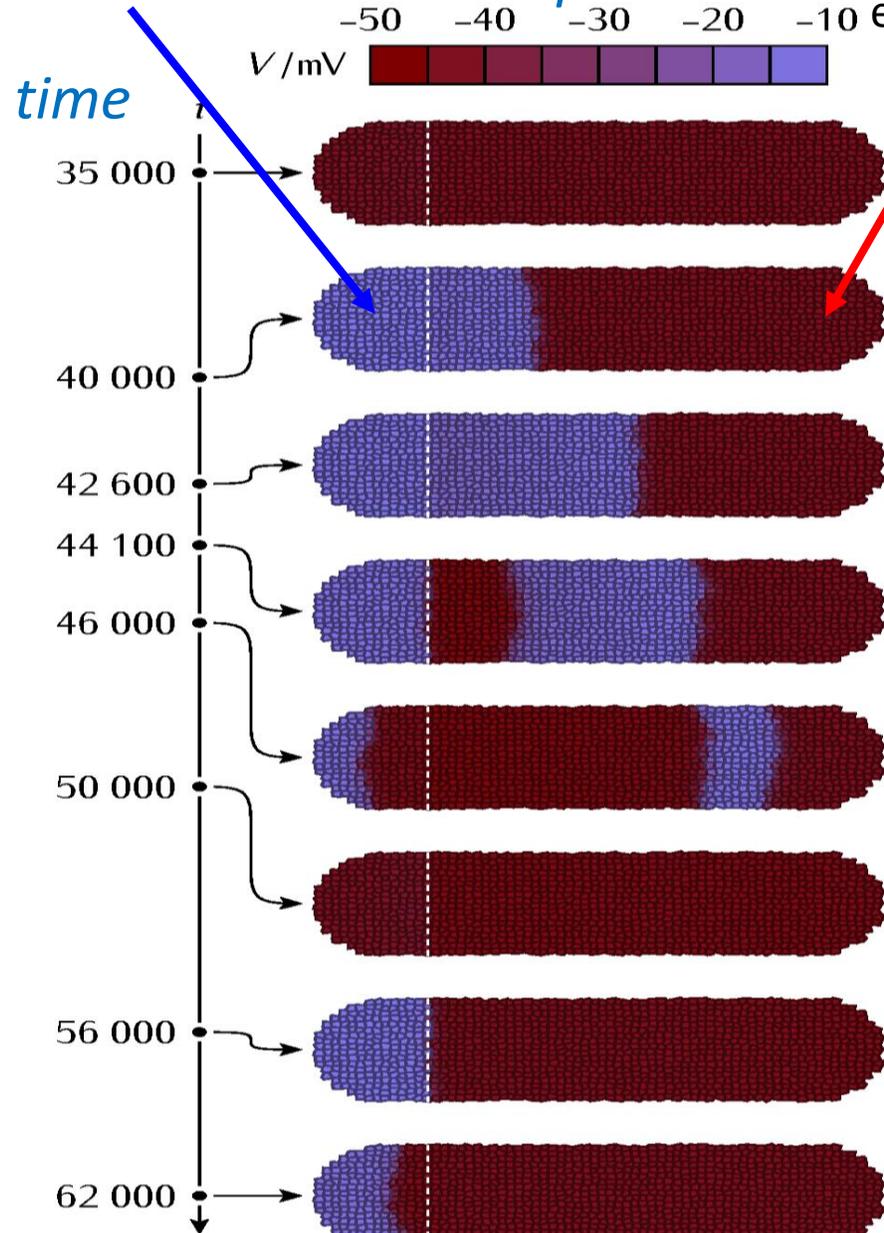
increasing intercellular connectivity →



**Synchronization phenomena:** the modulation of the *junction conductance* can establish *regions* with the same *oscillatory phase* of *bioelectricity* and *transcription* (left).

A *biochemical* reminiscence?: when the tissue is dissociated, cells stop oscillating, but coordinated oscillations arise by re-aggregation (bottom).





*symmetry breaking amplified: bioelectrical-transcriptional waves*

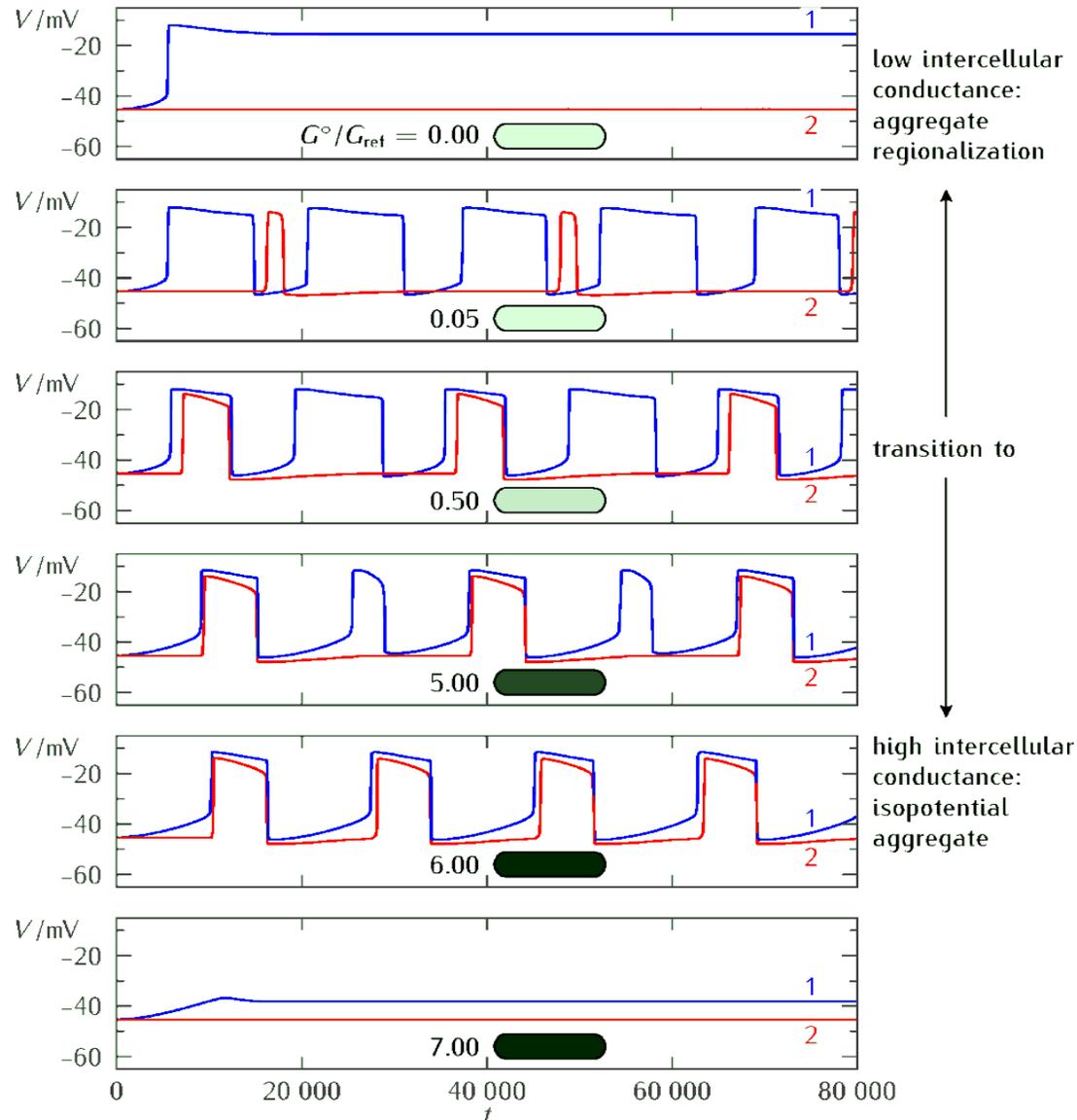
A *depolarization wave* propagates across the multicellular system evidenced by the *spatio-temporal bands* of alternate *dep* and *pol* regions.

Could the coexistence of *phase* and *antiphase regions* be *developmentally* relevant?

A *polarized central region* between two depolarized neighboring regions can *arise* and *expand* at long times.

The *coupling* between *bioelectricity* and *transcription* can encode *spatio-temporal information* (e.g. segmentation) through the *response* of *multicellular potentials* to a *symmetry-breaking* (the gradient of *pol* channel expression here).

# transitions between bioelectrical states regulated by intercellular gap junctions

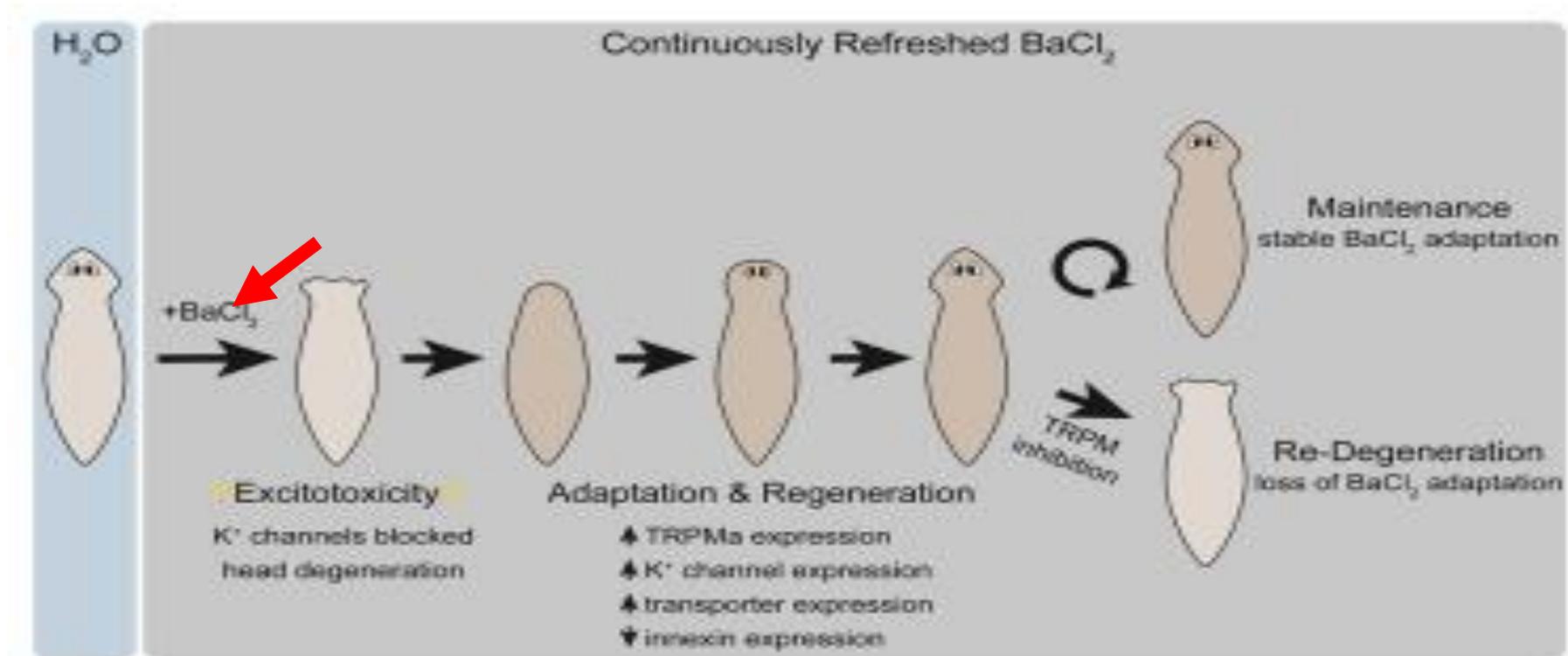


At *zero connectivity* (top), the *depolarized* (cell # 1, left region) and *polarized* (cell # 2, right region) are *independent* and show different potentials. *Trivial two-region encoding*.

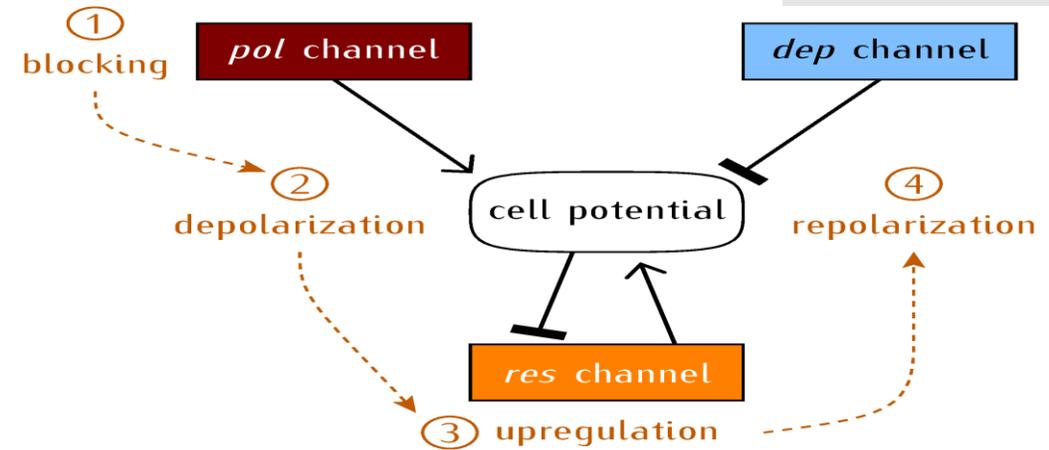
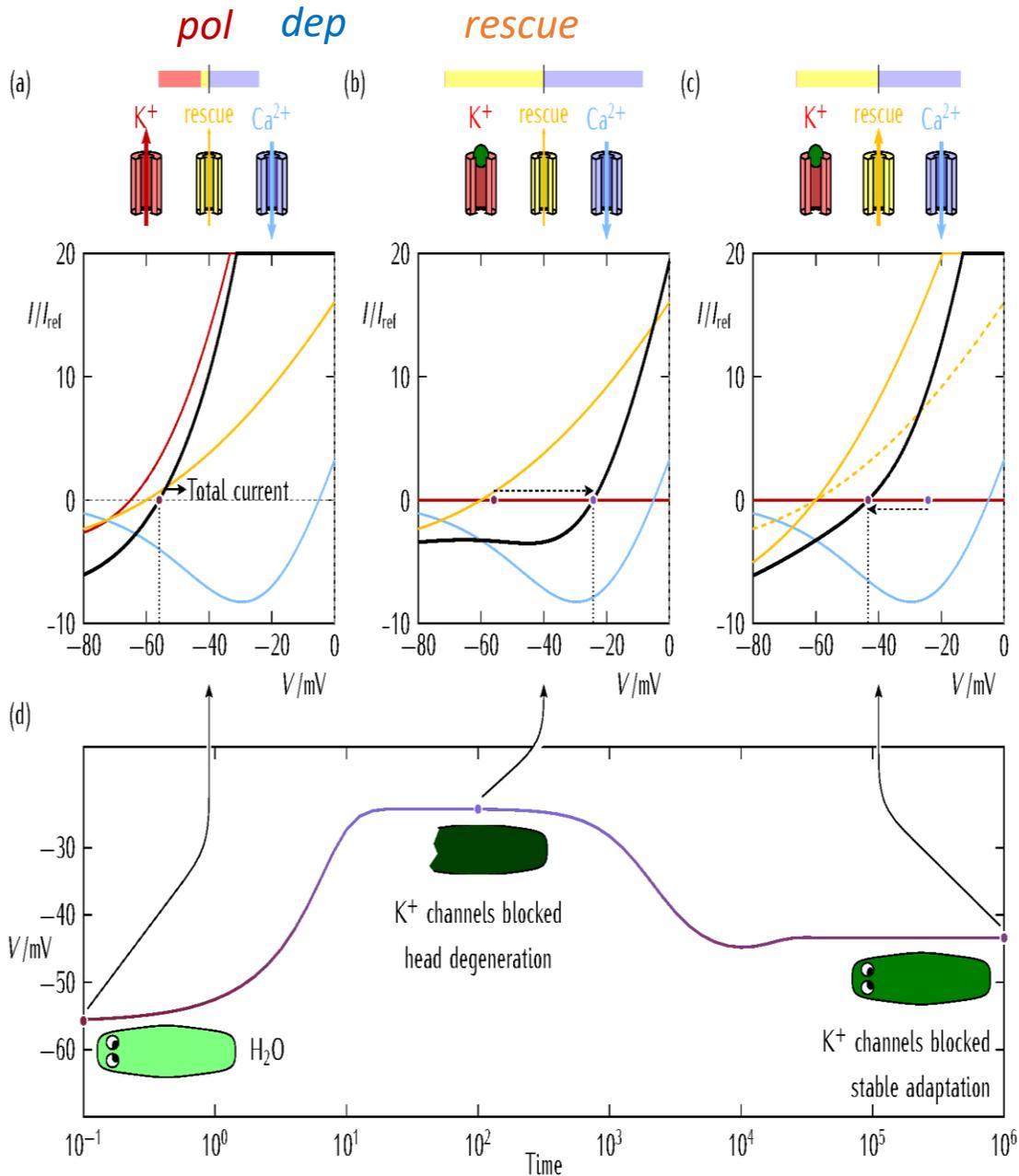
*Intermediate connectivity* couples the *left* and *right* regions in *phase* and *antiphase* oscillations, leading to a rich dynamics and the eventual *synchronization* of *distant* cell potentials: a *developmentally-relevant* case?

At *high connectivity* (bottom), an homogenous *iso-potential* system is obtained: *nothing can be encoded*.

## planarian regeneration as a model for acquired tolerance to toxic environments

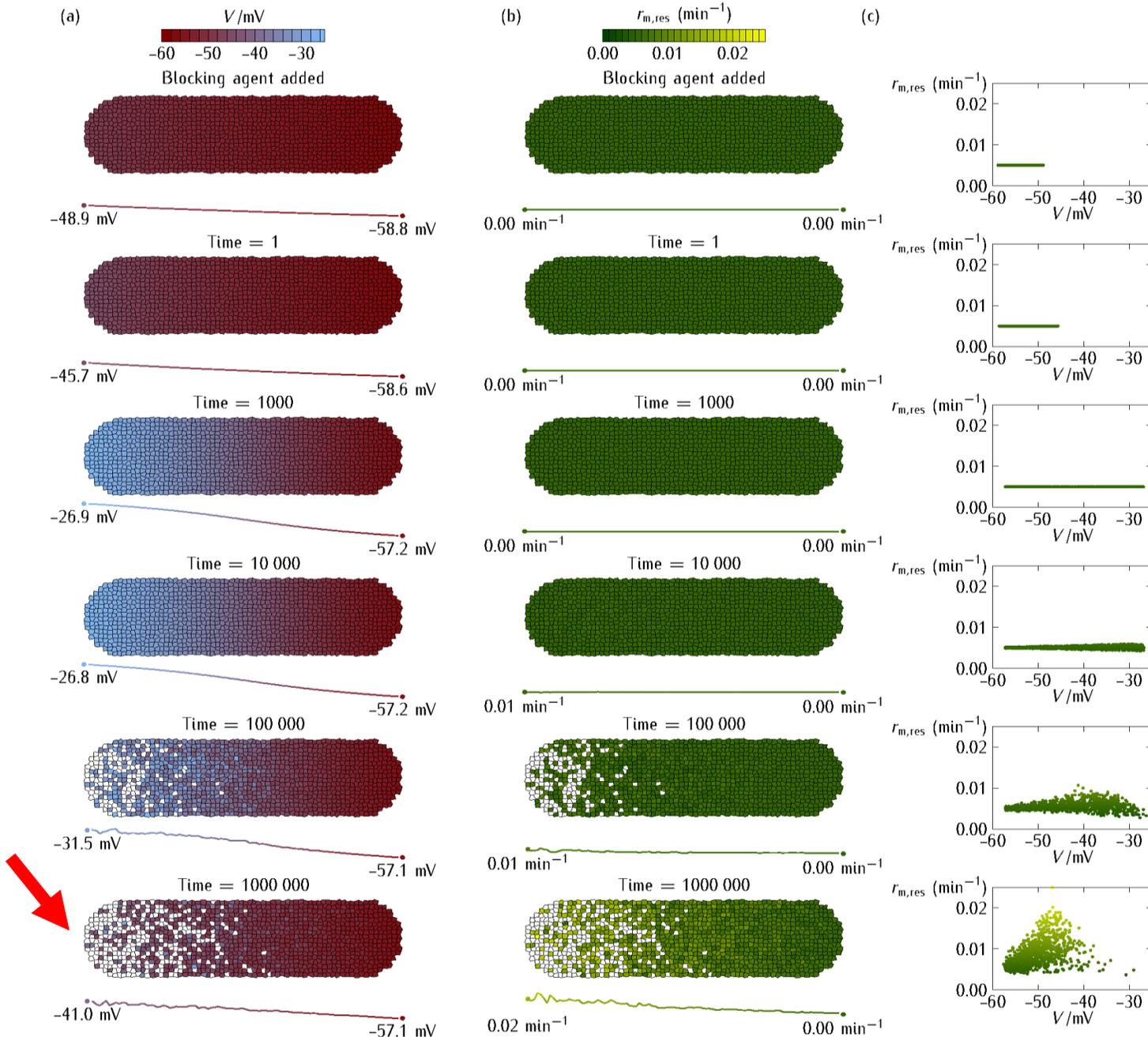


Exposure to barium chloride (BaCl<sub>2</sub>) provokes a rapid degeneration of anterior tissue in *Dugesia japonica*. However, continued exposure to fresh solution of BaCl<sub>2</sub> results in regeneration of heads that are insensitive to BaCl<sub>2</sub>. A model of *adaptation to toxicity* shows the central role of *ion channels* in *adaptive plasticity*, which may provide a target for *biomedical* strategies.



### Bioelectrical model: ion channels and cell polarization

- The  $I$ - $V$  curves of two opposing channels show the outward (*red*) and inward (*blue*) currents by the potassium and calcium channels before blocking.
- When the potassium channel is blocked, the depolarized cell potential marks the onset of the outward *rescue* channel.
- Cell physiological *repolarization* can eventually be achieved by the *increased expression* of the *compensatory rescue* channel.
- Time trace of the head cell potential.



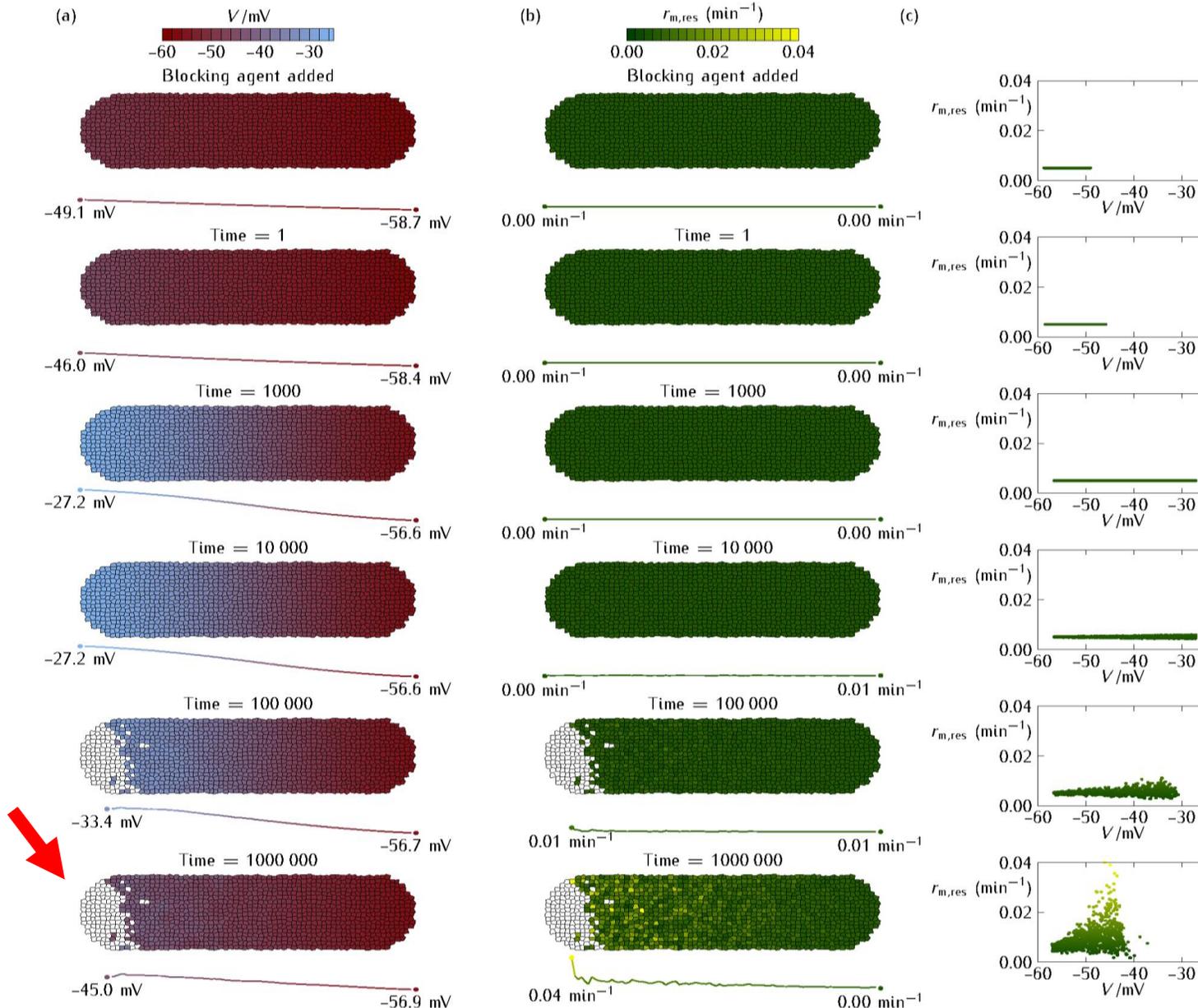
The single-cell state noisy updating can be *slightly biased* to upregulate the *res* channel that acts to reestablish cell polarization.

However, model simulations of the aggregate at *zero* junction conductance (isolated cells, *no* intercellular connectivity) show the *limited compensatory effect* of the *res* channel: note the significant *extension* of *dead* (white) *cells* in the head.

(a) Cell potentials  $V$ .

(b) Cell rate constants of the upregulated *res* channel.

(c) Rate vs.  $V$  plot for the living cells (points).



The model simulations of the multicellular aggregate at *non-zero* junction conductance (intercellular connectivity) show the significant *compensatory effect* of the *res* channel: note the *limited extension* of *dead* (white) *cells* in the head.

The result suggests a *collective* contribution to the *adaptive response* that may contribute to the bioelectrical *pattern completion* (head re-establishment here).

(a) Cell potentials  $V$ .  
 (b) Cell rate constants of the upregulated *res* channel.  
 (c) Rate vs.  $V$  plot for the living cells (points).



## Exploring Instructive Physiological Signaling with the Bioelectric Tissue Simulation Engine

Alexis Pietak and Michael Levin\*

Allen Discovery Center at Tufts University, Medford, MA, USA

Bioelectric cell properties have been revealed as powerful targets for modulating stem cell function, regenerative response, developmental patterning, and tumor reprogramming. Spatio-temporal distributions of endogenous resting potential, ion flows, and electric fields are influenced not only by the genome and external signals but also by their own intrinsic dynamics. Ion channels and electrical synapses (gap junctions) both determine, and are themselves gated by, cellular resting potential. Thus, the origin and progression of bioelectric patterns in multicellular tissues is complex, which hampers the rational control of voltage distributions for biomedical interventions. To improve understanding of these dynamics and facilitate the development of bioelectric pattern control strategies, we developed the BioElectric Tissue Simulation Engine (BETSE), a finite volume method multiphysics simulator, which predicts bioelectric patterns and their spatio-temporal dynamics by modeling ion channel and gap junction activity and tracking changes to the fundamental property of ion concentration. We validate performance of the simulator by matching experimentally obtained data on membrane permeability, ion concentration and resting potential to simulated values, and by demonstrating the expected outcomes for a range of well-known cases, such as predicting the correct transmembrane voltage changes for perturbation of single cell membrane states and environmental ion concentrations, in addition to the development of realistic transepithelial potentials and bioelectric wounding signals. *In silico* experiments reveal factors influencing transmembrane potential are significantly different in gap junction-networked cell clusters with tight junctions, and identify non-linear feedback mechanisms capable of generating strong, emergent, cluster-wide resting potential gradients. The BETSE platform will enable a deep understanding of local and long-range bioelectrical dynamics in tissues, and assist the development of specific interventions to achieve greater control of pattern during morphogenesis and remodeling.

**Keywords:** bioelectric simulation, pattern formation, resting potential, transmembrane voltage

### 1. INTRODUCTION

#### 1.1. Bioelectricity: Why Model Electrical Activity in Non-Neural Cells?

Explaining and learning to control large-scale pattern is a central unsolved problem, with implications for mitigation of birth defects, and the advancement of regenerative medicine and synthetic bioengineering. The dynamics of signals orchestrating large-scale order *in vivo* are a key area of

### OPEN ACCESS

#### Edited by:

Ovidiu Radulescu,  
Université de Montpellier 2, France

#### Reviewed by:

Victor P. Andreev,  
Arbor Research Collaborative for  
Health, USA  
Leonid Evsey Fridlyand,  
University of Chicago, USA

#### \*Correspondence:

Michael Levin  
michael.levin@tufts.edu

#### Specialty section:

This article was submitted to  
Bioinformatics and Computational  
Biology, a section of the  
journal Frontiers in Bioengineering and  
Biotechnology

Received: 10 April 2016

Accepted: 21 June 2016

Published: 06 July 2016

#### Citation:

Pietak A and Levin M (2016)  
Exploring Instructive Physiological  
Signaling with the Bioelectric Tissue  
Simulation Engine.  
Front. Bioeng. Biotechnol. 4:55.  
doi: 10.3389/fbioe.2016.00055

## INTERFACE

rsif.royalsocietypublishing.org

### Research



Cite this article: Pietak A, Levin M. 2017

Bioelectric gene and reaction networks:  
computational modelling of genetic,  
biochemical and bioelectrical dynamics  
in pattern regulation. *J. R. Soc. Interface* 14:  
20170425.  
<http://dx.doi.org/10.1098/rsif.2017.0425>

Received: 8 June 2017

Accepted: 31 August 2017

#### Subject Category:

Life Sciences – Physics interface

#### Subject Areas:

biophysics, computational biology,  
systems biology

#### Keywords:

bioelectricity, regeneration, gene regulatory  
networks, *in silico* simulations

#### Author for correspondence:

Michael Levin  
e-mail: michael.levin@tufts.edu

Electronic supplementary material is available  
online at <https://dx.doi.org/10.6084/m9.figshare.c.3379404>.

THE ROYAL SOCIETY  
PUBLISHING

## Bioelectric gene and reaction networks: computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation

Alexis Pietak and Michael Levin

Allen Discovery Center, Tufts University, Medford, MA, USA

ML, 0000-0001-7292-8084

Gene regulatory networks (GRNs) describe interactions between gene products and transcription factors that control gene expression. In combination with reaction–diffusion models, GRNs have enhanced comprehension of biological pattern formation. However, although it is well known that biological systems exploit an interplay of genetic and physical mechanisms, instructive factors such as transmembrane potential ( $V_{\text{mem}}$ ) have not been integrated into full GRN models. Here we extend regulatory networks to include bioelectric signalling, developing a novel synthesis: the bioelectricity-integrated gene and reaction (BIGR) network. Using *in silico* simulations, we highlight the capacity for  $V_{\text{mem}}$  to alter steady-state concentrations of key signalling molecules inside and out of cells. We characterize fundamental feedbacks where  $V_{\text{mem}}$  both controls, and is in turn regulated by, biochemical signals and thereby demonstrate  $V_{\text{mem}}$  homeostatic control,  $V_{\text{mem}}$  memory and  $V_{\text{mem}}$  controlled state switching. BIGR networks demonstrating hysteresis are identified as a mechanism through which more complex patterns of stable  $V_{\text{mem}}$  spots and stripes, along with correlated concentration patterns, can spontaneously emerge. As further proof of principle, we present and analyse a BIGR network model that mechanistically explains key aspects of the remarkable regenerative powers of creatures such as planarian flatworms. The functional properties of BIGR networks generate the first testable, quantitative hypotheses for biophysical mechanisms underlying the stability and adaptive regulation of anatomical bioelectric pattern.

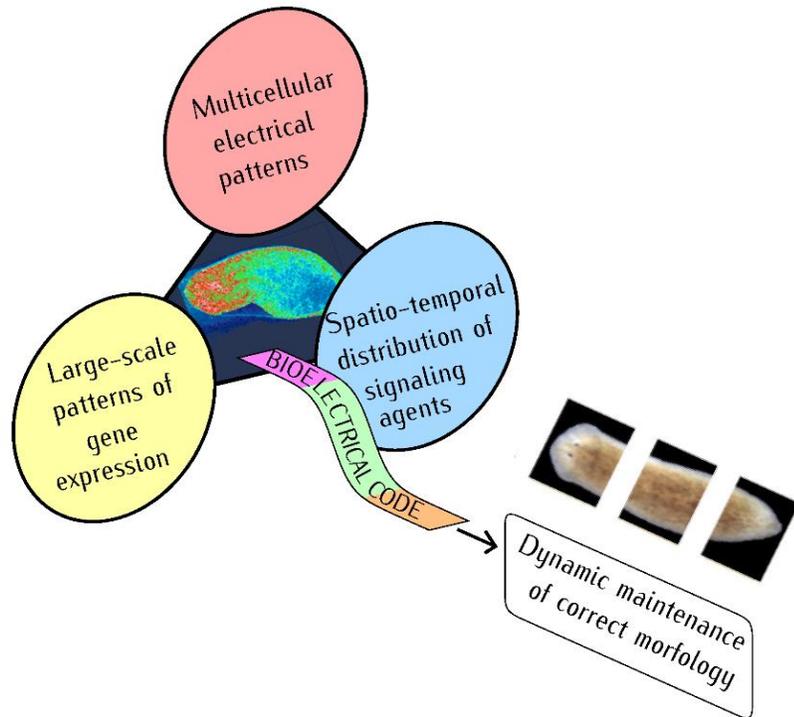
### 1. Introduction

Large-scale biological patterning in development, regeneration and disease remains among the most fundamental and important questions facing modern biology. Metazoan organisms reliably self-assemble a complex body plan from a single fertilized egg cell; furthermore, many animals, such as salamanders and planaria, are able to repair or remodel their bodies back to the correct shape despite injury and other types of drastic perturbation such as limb amputation [1,2]. Understanding the mechanisms that control the formation and regulation of organism-scale biological patterns may allow us to mitigate birth defects, implement organ regeneration strategies and to prevent, heal or even reprogramme the cancer state [3]. It is crucial to begin to understand and exploit the multicellular algorithms and dynamics that control anatomy and its remodelling, in addition to the details of subcellular signalling pathways.

Biological pattern formation is highly complex, involving numerous biomolecular mechanisms that lead to formation of instructive chemical patterns in a tissue collective, as well as mechanical considerations concerning shape changes and movements of individual cells and the tissue substratum as a whole. From the chemical patterning perspective pioneered by Turing [4] and Wolpert [5] (among others [6–8]), individual cells produce a variety of substances which may: (i) have the capacity to influence the production of other substances via genetic expression

## 6. Where we go: identifying key bioelectrical steps in biological complexity

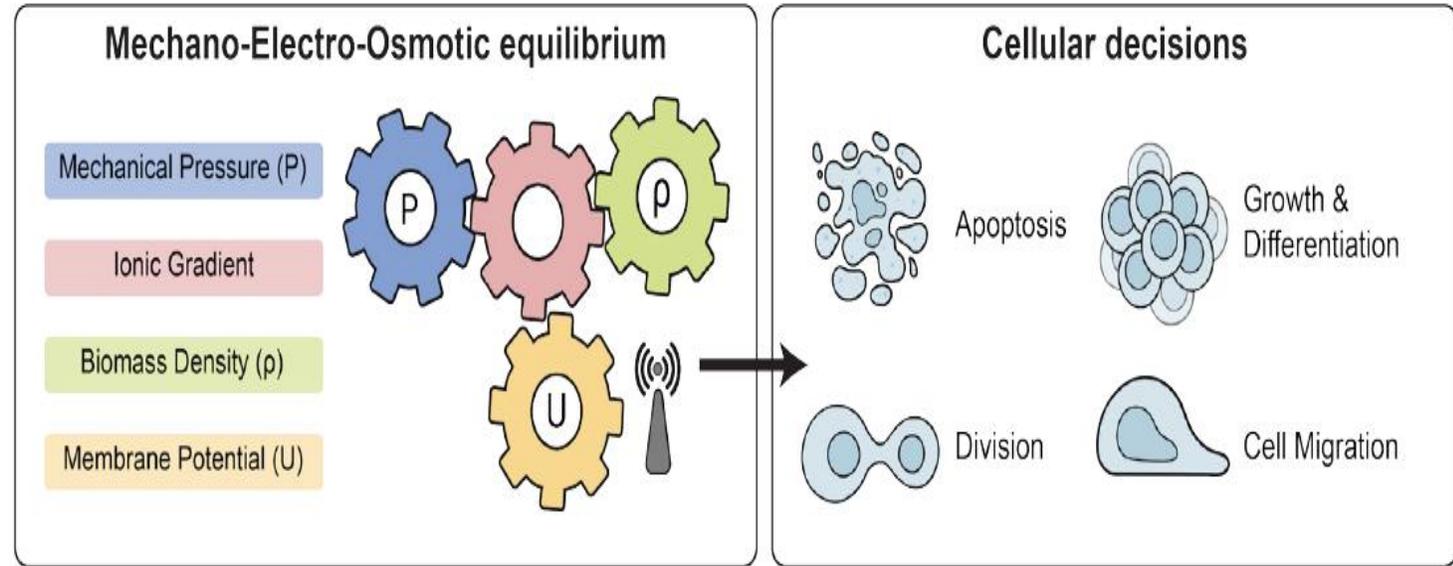
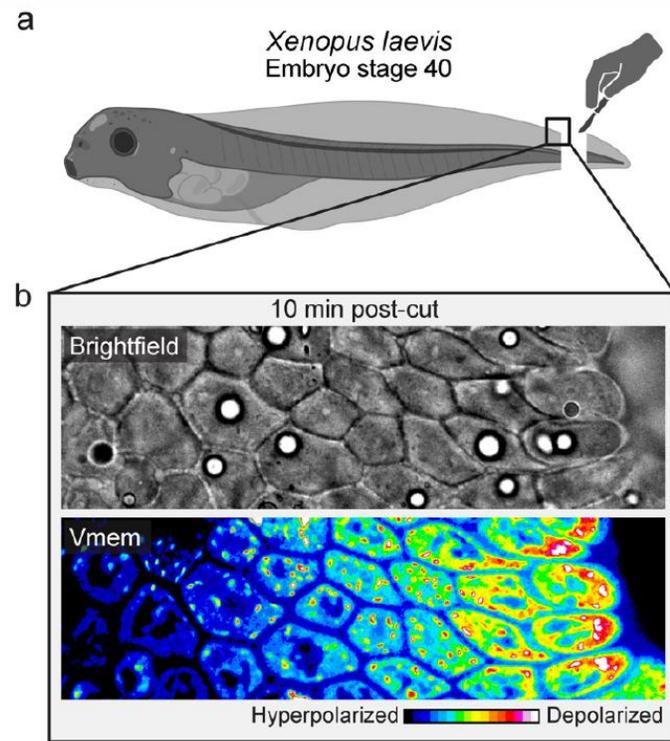
### 6.a Bioelectrical limitations



The model simulations explore the *interplay* between *transcription* and *bioelectricity*, modulated by the *single-cell states* and the *intercellular coupling*, in the establishment of *instructive* spatio-temporal patterns at the *multicellular level*. Could *perturbative* changes be *suppressed* or *enhanced* by interrelated *bioelectrical* and *biochemical* processes?

Although the physical model is exceedingly *simple* for quantitative descriptions, it suggests a *rich* phenomenology, based on the *multicellular coupling* between *bioelectricity* and *transcription*, that can be explored further. Also, the core concepts are general enough to permit future *extensions*.

In moving forward, however more *realistic* models must incorporate *additional contributions* to morphogenesis/regeneration, together with the *coordinated integration* of controlling signals. In particular, concerted *bioelectrical-biomechanical-biochemical* actions should act together to modulate expression patterns, as shown in the following cases.



*biomechanical pressure, cytoplasmic biomass density and membrane potential*

The *interplay of membrane potential and cytoplasmic dilution* at the initiation of *Xenopus* tail regeneration after amputation (a) and the membrane potential (DiSBAC2(3) dye) images of the wound edge (b) suggest that cell layers are progressively depolarized, from the deep tissue towards the wound edge.

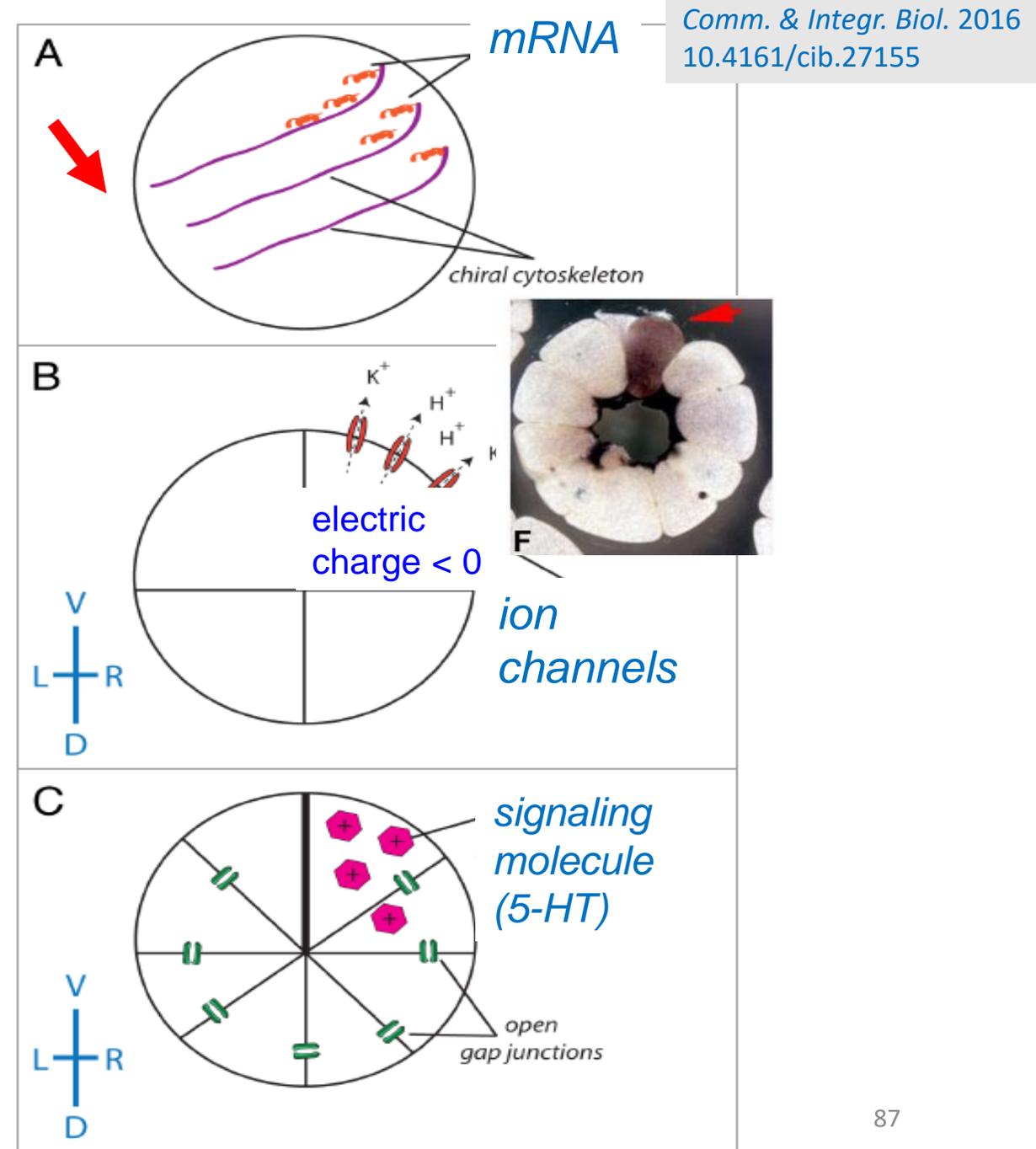
*mechano-transduction, bioelectricity and biochemical networks acting together in Xenopus frog model*

(A) Cytoskeleton *asymmetrically* distributes *mRNAs* encoding *ion transporters*.

(B) The biased expression gives an *electric potential* difference.

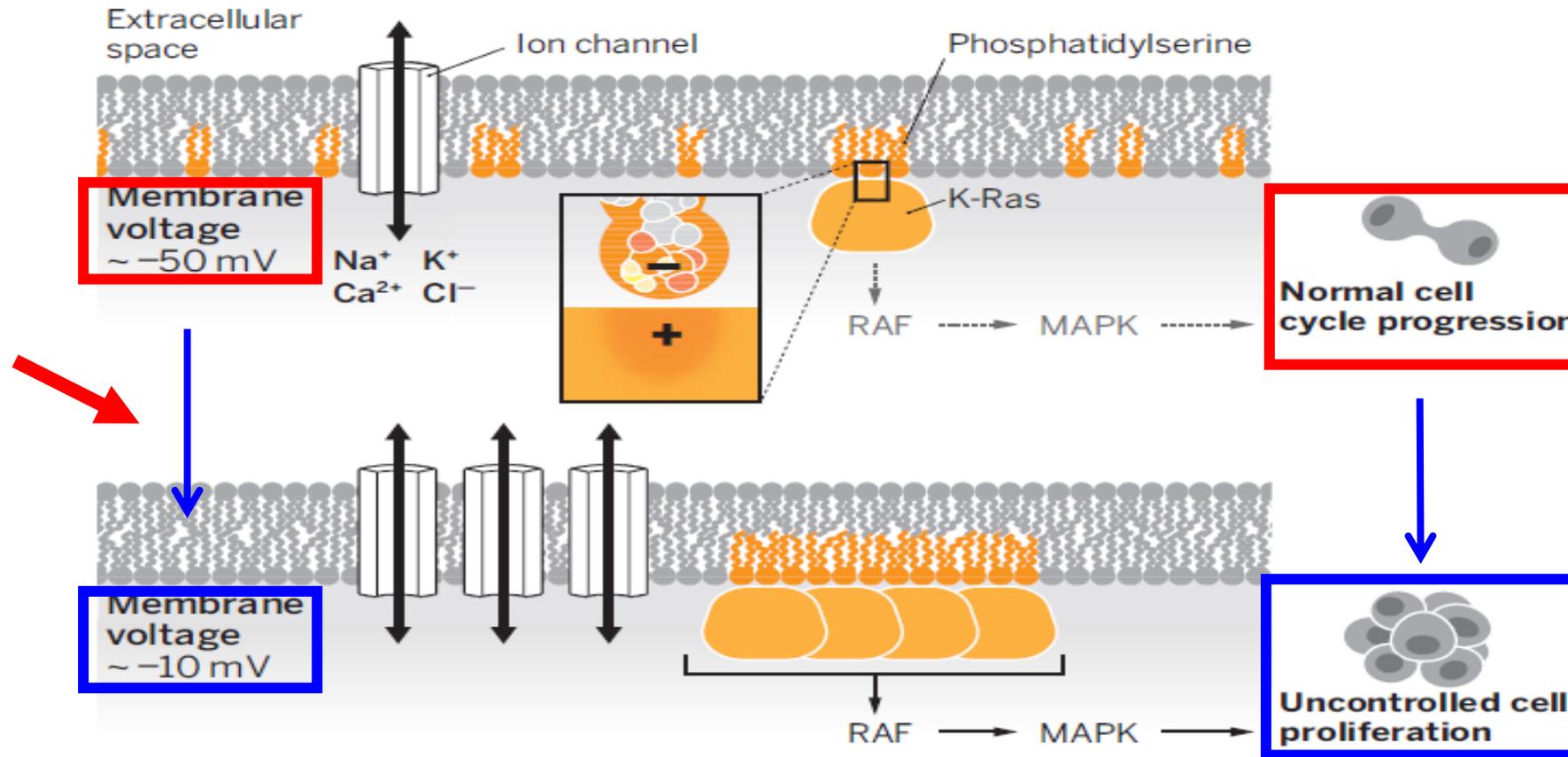
(C) Negative potential in the ventral right blastomere yields a *net flow* of positively charged *signaling molecules* (serotonin).

The *electric potential* regulates the spatial distribution of *signaling molecules* and *ions* over multicellular domains but *biomechanical* effects are also present.



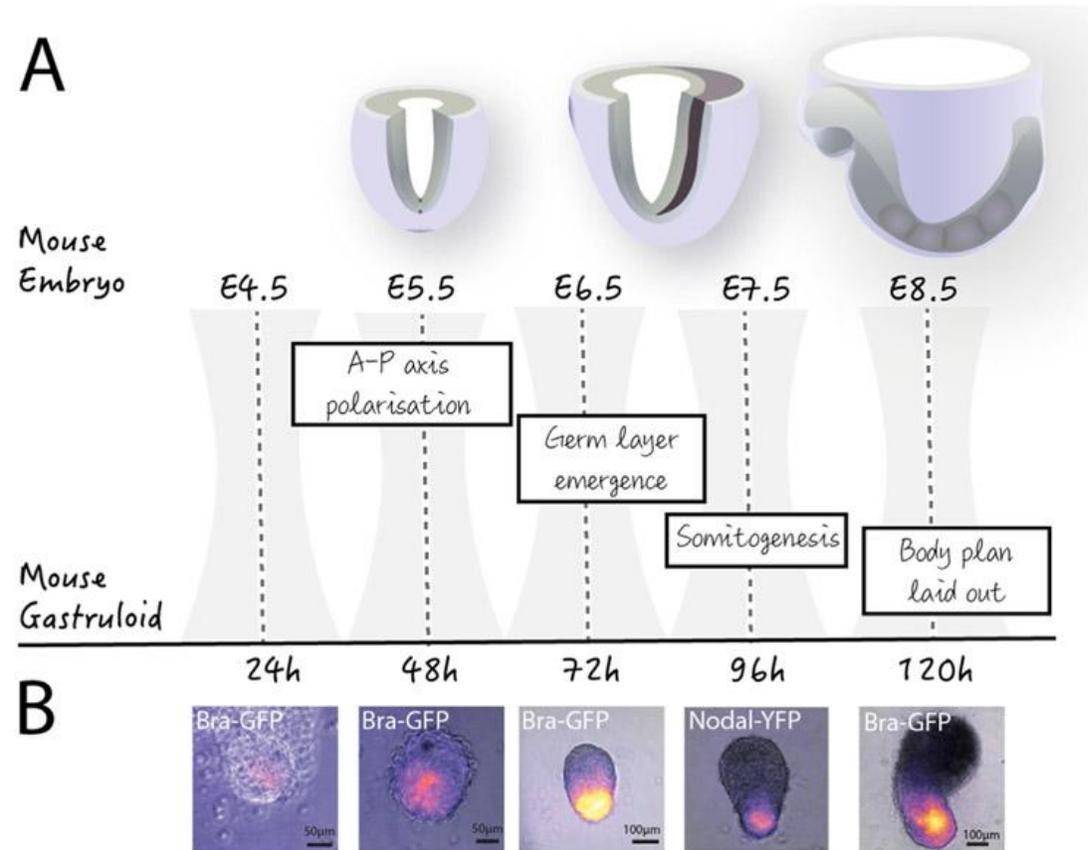
# bioelectricity, biomechanics, and biochemistry are coupled in tumorigenesis

Science 2015  
10.1126/science.aaa5619  
10.1126/science.aad0874  
eLife 2024  
10.7554/eLife.101613.1



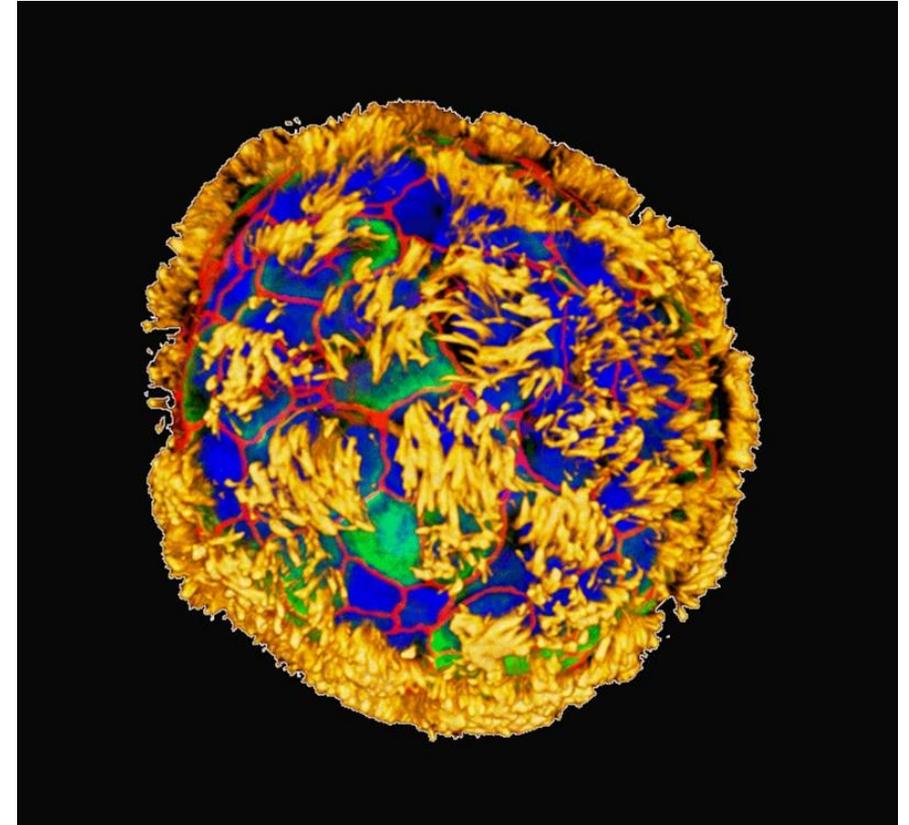
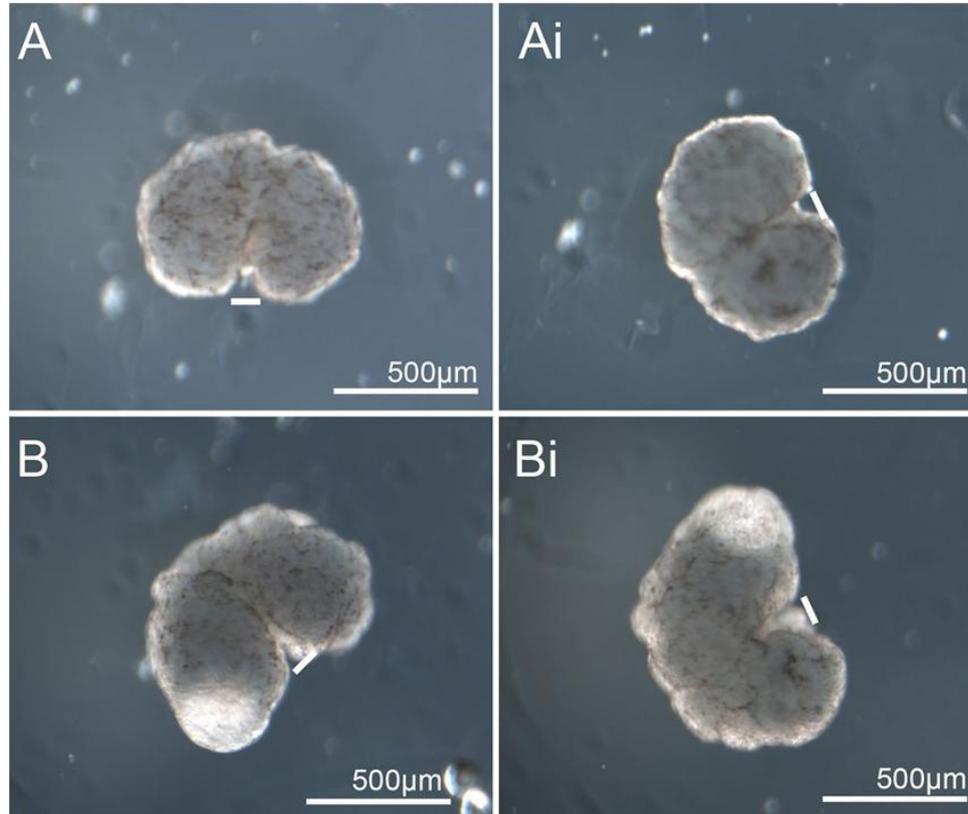
*Cell proliferation and physicochemical properties of lipids: the membrane as a biomechanical field-effect transistor.* Other studies consider the extracellular signal regulated kinase (ERK) as relevant for cell potential and proliferation.

# gastruloid biomechanics: pluripotent stem cell models of mammalian gastrulation and embryo engineering

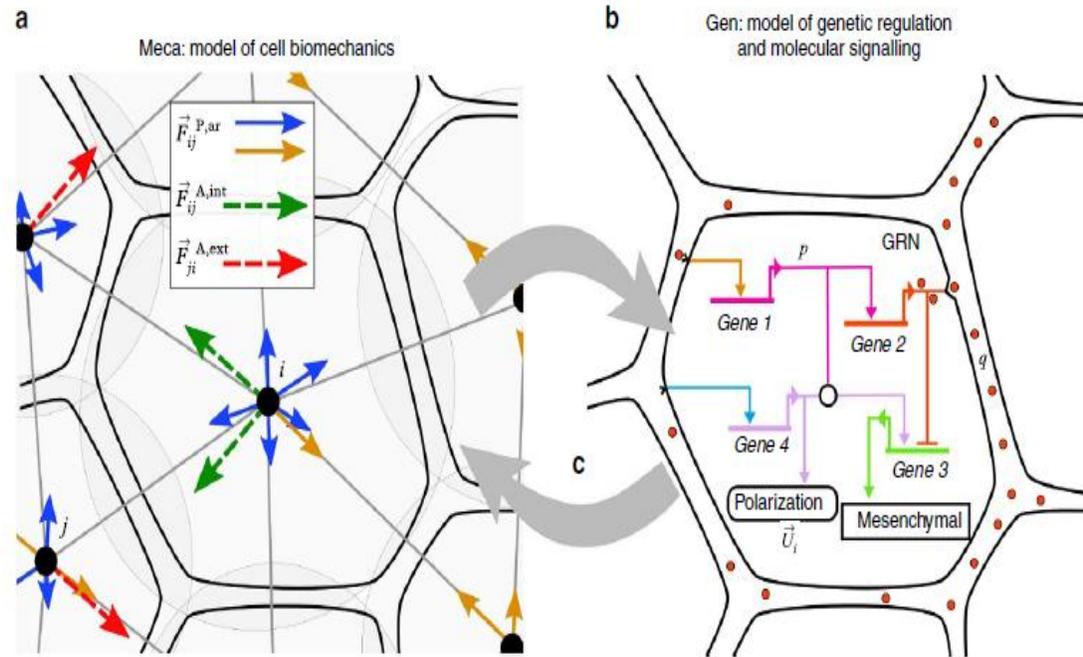


Relationship between mouse embryo (A) and gastruloid (B) development. Gastruloids and polarization of *Bra* and *Nodal* gene expression. The regulation of the fate of *stem cells* and *biomechanical field* effects influence the target shape.

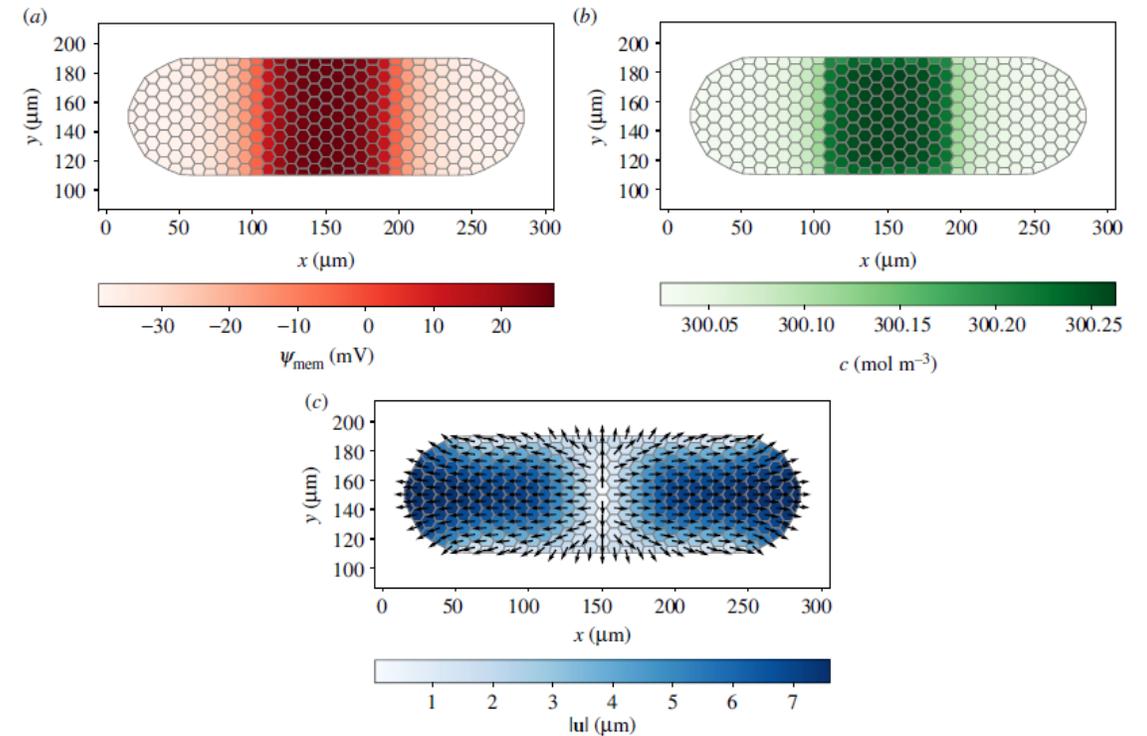
*biobots: what are the multicellular biomechanical fields that govern what cells can do besides create default features in the body?*



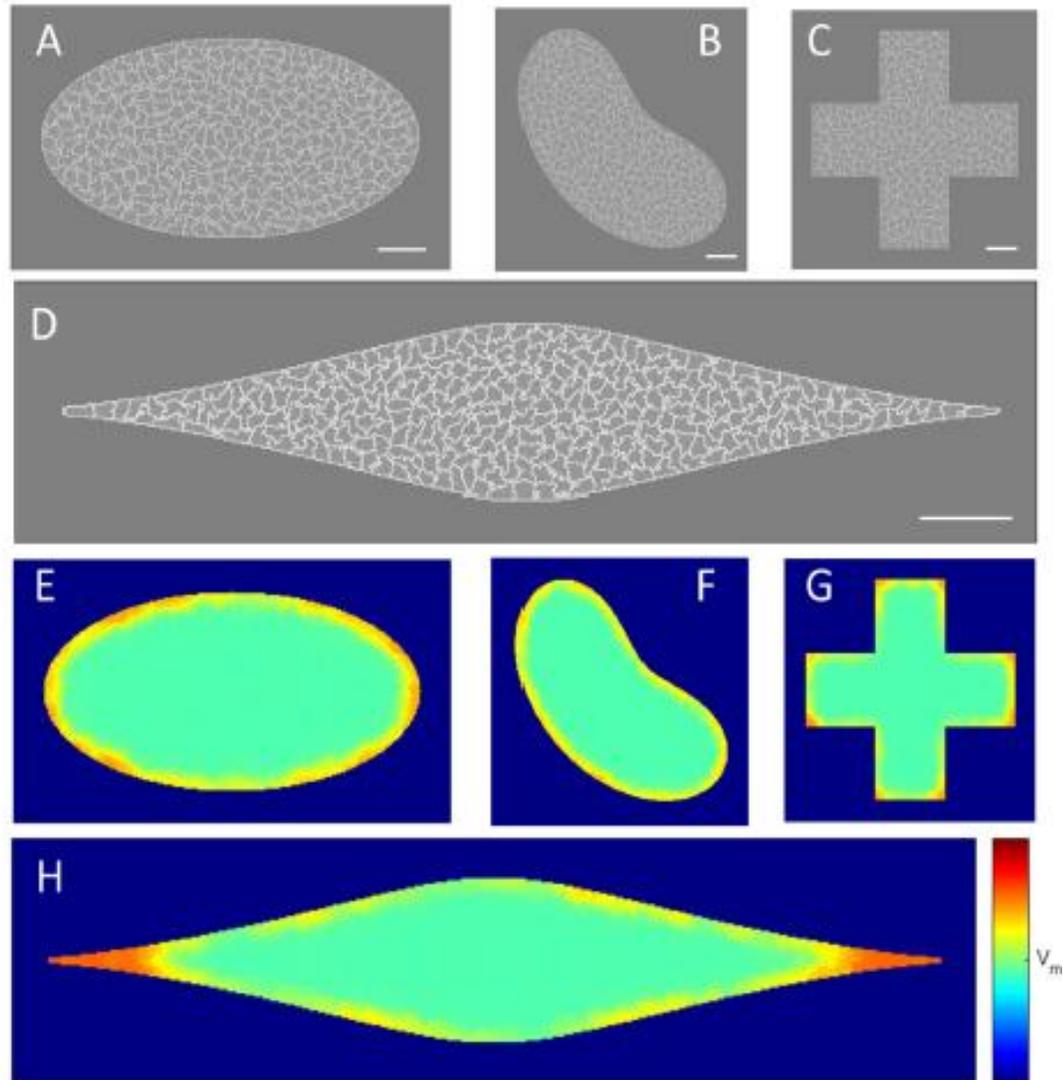
# coupling biomechanical, bioelectrical, and biochemical models



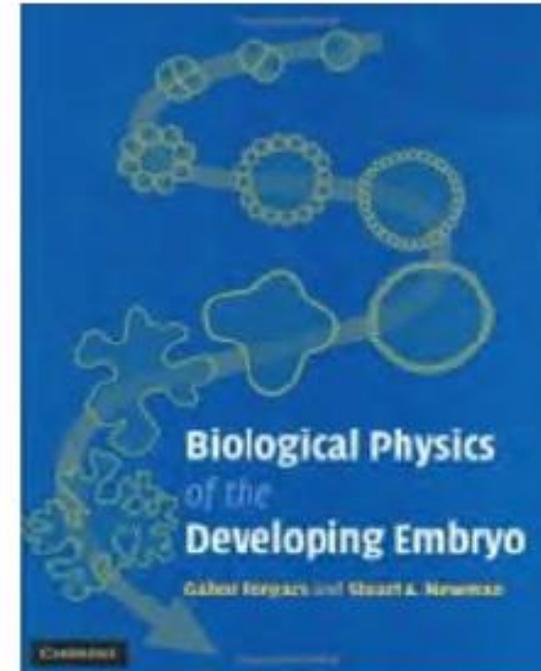
Schematic overview of the *MecaGen* model *coupling* the cell *biomechanical* properties to its *biochemical* activity. Mechanical parameters are specified by the gene expression dynamics and molecular state. Conversely, spatial rearrangements among cells impact protein synthesis via signaling and mechanical stress.



Modeling the *coupling* of *mechanics* with *bioelectricity* and its role in *morphogenesis*: cell membrane potential (a), osmotic concentration (b) and displacement vector (c) at a fixed time. The central depolarized region in which ions accumulate determines a symmetric horizontal elongation of the cluster.



*Shape* effects matter: simulated average *multicellular potentials*  $V_m$  in *different geometries*: elliptical, bean-like, cross, and eye-like (A to H).

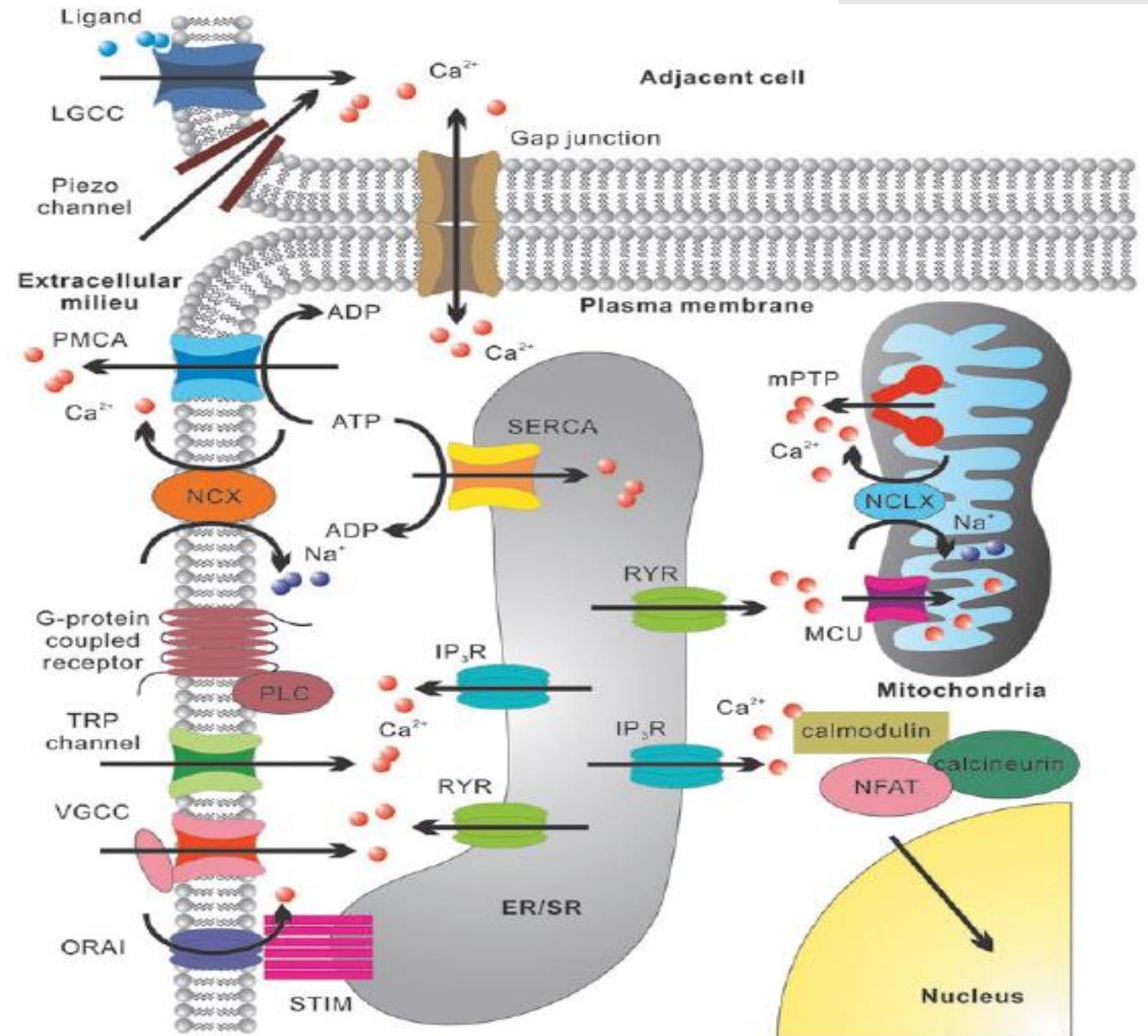


*Biological Physics of the Developing Embryo*  
Gabor Forgacs and Stuart A. Newman.  
Cambridge University Press, 2005. *Biomechanical*  
compartment formation and gastrulation are  
central in *morphogenetic* processes.

*multitude of ion transporters, not only voltage-gated channels, can establish bioelectrical and biochemical feedbacks*

*Biological complexity:* schematic representation of key regulators of cytosolic  $\text{Ca}^{2+}$  levels.

*Important:* extensions of the bioelectrical model must be considered in *real cases*.



## 6.b Bioelectrical opportunities

- 1) The progress from an *individual cell* to *multicellularity* leads to *average* biophysical magnitudes. They constitute *higher levels of abstraction* that provide an actuation layer *complementary* to cell-level management. A *data-driven bioelectrical view*, complementary to biochemical and biomechanical models, can provide *qualitative insights* into bioelectricity and transcription: reduce complexity by identifying those magnitudes relevant to *average multicellular potentials*.
- 2) Main features of *multicellular fields –a Statistical Physics* view:
  - basic *units* need *not* be *equal* for a *robust collective response*: individual heterogeneity, which allows the *diversity* needed to respond to environmental changes, can be compensated by intercellular *connectivity* and *redundancy* mechanisms;

- *multicellular fields* can manage individual *variability* and *environmental noise*, avoiding tight regulation at the single-cell level. Also, *low-dimension average responses* tend to demand *small system resources* compared with an exhaustive control of every unit;
- *transitions* between *instructive multicellular patterns* can be obtained by *local remodeling* of the *intercellular connectivity*; while this fact may increase system *vulnerability*, it can also allow external *correcting actions*; and
- “*programming at the bottom layer with uncertain consequences on upper layers*”: if morphogenetic processes are *complex to micromanage*, the control of *average multicellular fields* could offer future opportunities.

3) The *Bioelectricity* of non-neural tissues is an *additional regulatory pathway* that can be modulated by molecular-genetics, pharmacological, and optogenetical techniques to *open* and *close* the ion channels and gap junctions. The *bioelectric concepts* introduced here suggest particular hypotheses that could be tested by *reductionist procedures* in *model systems*.

### *Important:*

The *integration of Bioelectricity* with biological principles is still *in progress*, with some unconventional but potentially useful results. A significant *limitation* is that *external actions* are *system-dependent* and *context-sensitive* because of the *single-cell and multicellular feedbacks* between channels and junctions. Predictive power could be increased by further generalization of initial findings.

Ion channels and intercellular junctions regulate *multiple functions* such as cardiac rhythm, muscle contraction, and neurological functions, being involved in crucial cell properties (adhesion, volume regulation, apoptosis) at *different levels* of biological organization. Thus, a detailed knowledge of the *target* and *anti-target* channels and possible feedback mechanisms is required to implement any bioelectrical action; with the *risk of unknown* non-canonical channel functions that can lead to *unintended* and *adverse events*.

*Cell* 2021  
10.1016/j.cell.2021.02.034  
*Biochim. Biophys. Acta* 2023  
10.1016/j.bbagen.2023.130440  
*Bioelectricity* 2022  
10.1089/bioe.2022.0014  
*Annu. Rev. Cell Dev. Biol.* 2015  
10.1146/annurev-cellbio-100814-125338  
*Development* 2021  
10.1242/dev.180794

Thanks to:

Patricio Ramirez

Vaibhav P. Pai

Alexis Pietak

José A. Manzanares



Ministerio de Ciencia e Innovación (PID2022-139953NB-I00)



Conselleria d'Educació, Universitats i Ocupació (CIAICO2022-247)

**The Journal of Chemical Physics**  
 AIP Publishing  
 Vol. 162, Iss. 19, 21 May 2025

**Characterization of negative differential resistance in asymmetric nanopores obtained from two soluble electrolytes**  
 Patricio Ramirez, Sergio Portillo, Salvador Mafe, Zuzanna S. Sivy, and Javier Cervera

Available Online: [pubs.aip.org/jcp](https://pubs.aip.org/jcp)

**THE JOURNAL OF PHYSICAL CHEMISTRY B**  
 MAY 9, 2019  
 VOLUME 123  
 NUMBER 18  
[pubs.acs.org/JPCB](https://pubs.acs.org/JPCB)

ACS Publications  
 Most Trusted. Most Cited. Most Read.  
[www.acs.org](http://www.acs.org)

**Bioelectrochemistry**  
 Formerly Bioelectrochemistry and Bioenergetics  
 Volume 123 October 2016 ISSN 1567-5304

An International Journal Devoted to Electrochemical Aspects of Biology and Biological Aspects of Electrochemistry  
 Official Journals of the Bioelectrochemical Society

Editor in Chief:  
**P. Vadgama**

Available online at: [sciencedirect.com](https://www.sciencedirect.com)  
 ScienceDirect

**PROGRESS IN BIOPHYSICS & MOLECULAR BIOLOGY**  
 An International Review Journal  
 Volume 148 | December 2018 | ISSN 0079-6187

EDITORS  
 DENIS NOBLE  
 TOM L. BLUNDELL  
 PETER KOHL

Available online at: [sciencedirect.com](https://www.sciencedirect.com)  
 ScienceDirect